The Prevalence and Risk Factors in Associated to Antibiotic Resistance of Bacteria from Diarrhoeal Patients in Bac Ninh Hospital Northern Viet Nam

Student: Ngo Tuan Cuong

Supervisor:

Gunnar Bjune, Professor, M.D., Ph.D.,

Department of International Health,
Institute of General Practice and Community Medicine.
University of Oslo - Norway

Co - supervisor:

Nguyen Binh Minh, Ass. Professor, M.D., Ph.D.,

Department of Bacteriology
National Institute of Hygiene and Epidemiology
Hanoi - Vietnam.

Faculty of Medicine
Department of General Practice and Community Medicine
Section for International Health
June 2005

UNIVERSITY OF OSLO

NIHE HA NOI, VIET NAM

Thesis submitted as a part of the
Master of Philosophy in International Community Health

CONTENTS

LIST OF TABLES AND FIGURES

TABLES

FIGURES

ABBREVIATIONS

AM:	ampicillin
CDC:	Centers for Disease Control and Prevention
CI:	Confident Interval
CIP:	ciprofloxacin
CL:	chloramphenicol
CRO:	ceftriaxone
DNA:	Dezoxyribo nucleic acid
DOC:	Drug of choice
EAggEC:	enteroaggregative *Escherichia coli*
E.coli:	*Escherichia coli*
E.faecium:	*Enterococcus faecium*
EHEC:	enterohemorrhagic *Escherichia coli*
EIEC:	enteroinvasive *Escherichia coli*
EPEC:	enteropathogenic *Escherichia coli*
EPI:	Expanded Programme for Immunisation
ETEC:	enterotoxigenic *Escherichia coli*
EU:	European Union
FDA:	Food and Drug Administration
GDP:	Gross domestic product
LT:	heat-labile toxin
MDR:	Multidrug resistance
MIC:	Minimal inhibitory concentration
MOH:	Ministry of Health
NIHE:	Natinal Institute of Hygiene and Epidemiology
OR:	Odds ratio

PCR:	Polymerase chain reaction
spp.:	species
SPSS:	Software Package for Social Sciences
S.typhi:	*Salmonella typhi*
ST:	heat-stable toxin
SXT:	trimethoprim-sulfamethoxazol
TE:	tetracyclin
USA:	United States of America
USD:	United States dollar

ABSTRACT

Background: Antibiotic resistance has become a worldwide problem. Recently, there has been a global increase in infections caused by microorganisms resistant to multiple antibiotics. This has led to increases in morbidity and mortality and increased the cost of health care, which threatens to become unaffordable in developing countries. Behavioural factors, particularly the misuse of antibiotics; and lack of infection control practices in communities are the most common factors which lead to increase in antibiotic resistance. In Viet Nam, self-treatment is very common. Inappropriate use of antibiotics is frequent in the communities and in the hospitals. The development of antibiotic resistance is increasing in Viet Nam. Self-medication has been associated, in particular, with the overuse and inappropriate use of antibiotic drugs, and this has caused antibiotic resistance levels in Viet Nam to reach epidemic levels. This research was carried out in Bac Ninh province in order to identify the risk factors contributing to antibiotic resistance of enteric bacteria.

Objective: The objectives of this study were identifying the risk factors that contribute to antibiotic resistance of bacteria; studying the pattern of use of antibiotics in the community; and determining the antibiotic susceptibility of enteropathogenic *Echerichia coli* (EPEC) strains isolated from the diarrhoeal patients.

Methods: A hospital based cross-sectional study was performed. Suspected cases were diarrhoeal patient with greater than or equal to three diarrhoeal movements per day, who were admitted to Bac Ninh hospital from August to December, 2004. Study's targets were defined as positive with EPEC in stool culture. Antibiotic sensitivity testing was performed on EPEC strains isolated. Exposure was defined as a case with EPEC strain resistant to at least one kind of the antibiotics tested. Non-exposure was defined as a case with EPEC strain sensitive to all of the antibiotics tested. Interviews were performed using a standard questionnaire collecting information regarding their behaviour concerning in antibiotic consumption, hygiene and socio-economic indicators.

Results: There were 227 cases positive with EPEC among the 526 diarrhoeal patients admitted to Bac Ninh hospital during period time of this study. Among

them, 171 cases were exposure and 48 cases were non-exposure (we did not include 8 cases, which were patients from outside of Bac Ninh Province). 68% of cases were children under 5 years of age. Following the conditional logistic regression model, being a patient with diarrhoea for more than 7 days (OR = 0.21; 95% CI: 0.05 - 0.93; P-value = 0.039); having a history of using antibiotic during the preceding 90 days (OR=5.41; 95% CI: 1.39 - 21.00; P-value = 0.015); having a low general knowledge (OR = 0.15; 95% CI: 0.04 - 0.52; P-value = 0.003); having used antibiotics in wrongly compared to medical instruction; and having been drinking un-boiled water were independently associated with antibiotic resistance of the EPEC strains isolated from the diarrhoeal patients.

Among the 179 cases (78.85%) resistant to at least one kind of antibiotic, 157 cases (69%) were resistant to two or more of the antibiotics. There was a high rate of resistance to ampicillin (69.16%) and trimethoprim/sulphamethoxazol (67.84%). In this study, we also found some EPEC strains (9 strains) resistant to ciprofloxacin.

Conclusion: Our study suggested that some factors such as: misuse of antibiotics; lack of knowledge about the signs of infectious disease; having a history of recently using antibiotics; drinking un-boiled water; and being exposed to unhygienic condition in living area were associated with antibiotic resistance of the disease – causing EPEC bacteria. There was a high proportion of resistance in the EPEC strains isolated from diarrhoeal patients. Antibiotic resistance has become an important problem. Multiple drug resistance (resistance with greater than or equal to two kinds of antibiotic) exists as a serious problem in Bac Ninh Province.

These findings suggest that monitoring and control of resistance to antibiotics of bacteria are very important. Antibiotic sensitivity testing in the health care centres (at least in the hospitals) should be carried out as a routine to ensure that suitable antibiotics are used for treating infectious diseases. Patient should follow the doctor's prescription. Over the counter sales of antibiotics should be stopped. Public communication is important to raise general knowledge of antibiotic use and encourage the population to use boiled water.

Key words: Risk factors, antibiotic resistance, EPEC, diarrhoea, Viet Nam, developing countries.

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to:

- Professor Gunnar Bjune, head of Department of International Health, Institute of General Practice and Community Medicine, University of Oslo, Norway, whose experience, constructive criticism, generous support and encouragement gave me energy, strength and self-confidence to finish this study.

- Associate Professor Nguyen Binh Minh, head of Bacteriology Department, National Institute of Hygiene and Epidemiology (NIHE), Ha Noi Viet Nam, for her support during the field work and her constructive comment on the study.

- Doctor Nguyen Thi Thanh Ha and all staffs in the Antibiotic Sensitivity Laboratory of NIHE for their stimulating support throughout the work with this thesis.

- Associate Professor Vu Tan Trao, head of Immunology and Molecular Biology Department; Professor Phung Dac Cam, head of Enteric Pathogens Research Unit, Microbiology Department - NIHE, Professor Hoang Thuy Long, Director of NIHE; for their recommendation to this course and their support during the study.

- The director and staffs of the Bac Ninh Hospital for their participation in this study. All patients, who decided to participate and answered the questionnaire in this study.

- All of my colleagues at Enteric Pathogens Laboratory, Microbiology Department, National Institute of Hygiene and Epidemiology, Hanoi Viet Nam, for helping and stimulating company.

- All of the staffs in the Department of General Practice and Community Health, (Faculty of Medicine, University of Oslo) as well as my friends and fellow students.

- My parents, my wife and my son for their love, encouragement, support and everything.

This study was supported by the Norwegian Agency for Development Cooperation (NORAD); section for International Health, Institute for General Practice and Community Medicine, University of Oslo and National Institute of Hygiene and Epidemiology, Ha Noi Viet Nam.

CHAPTER I: INTRODUCTION

1.1. Background

At the end of the 19th century, E. de Freudenreich, a German scientist isolated a bacterial secretion and noted its antibacterial properties [1]. At that time, earlier experiments had demonstrated interesting anti-bacterial effects from various bacterial secretions. Until the beginning of the 20th century infections were a major cause of morbidity and mortality in human beings. Diseases such as pneumonia, typhoid fever, diphtheria, dysentery, tuberculosis and malaria were common and lethal diseases. The introduction of antimicrobial and chemotherapeutic agents in the 1940s and 1950s has had a dramatic impact, improving the outcome and reducing the spread of many of these infectious diseases. However, at the same time, antibiotic resistance appeared. The first cases of antibiotic resistant bacteria were identified in the 1940s. While new antibiotics were being discovered at a steady rate the consequences of this phenomenon were slow to be appreciated. At the end of the 20th century the increase of resistance to these antimicrobial agents threatens to reverse their achievements. There is no doubt that misuse of antibiotics in human beings has contributed to the increasing rates of resistance [2,3].

The use of antimicrobial agents themselves does not cause resistance, but their presence creates a selective environment for resistant strains over their susceptible counterparts, allowing them to survive and to multiply. Equally important is the dissemination of these resistant micro-organisms that have evolved to the environmental.

Antibiotic resistance of bacteria has become a worldwide problem. The fundamental cause of the appearance and spread of antimicrobial resistance has been increasing antibiotics use. Several factors have lead to this increase, particularly the selection pressure exerted by use and misuse of antimicrobial agents. In recent years there has been a worldwide increase in infections caused by micro-organisms resistant to multiple antimicrobial agents. Antimicrobial resistance has led to increases in morbidity and mortality and increased the cost of health care, which threatens to become unaffordable in developing countries.

1.2. The mechanisms of antibiotic resistance

An antibiotic is a drug that kills or stops the growth of bacteria. Antibacterials are one class of "antimicrobials", a larger group which also includes anti-viral, anti-fungal, and anti-parasitic drugs. They are relatively harmless to the host, and therefore can be used to treat infection. The term originally described only those compounds derived from living organisms, but is now applied also to synthetic antimicrobials, such as the sulfonamides [4].

In treatment of infectious diseases, the antibiotics are drugs that target microbes without harming the host. Antibiotics are not effective in viral, fungal and parasitic infections, and individual antibiotics vary widely in their effectiveness towards various types of bacteria. Some specific antibiotics target either gram-negative or gram-positive bacteria; others are more wide-spectrum antibiotics. The effectiveness of individual antibiotics varies with the local of the infection and the ability of the antibiotic to reach this site. There are many ways to classify antibiotics. Chemical structure can be used to classify them. Another classification is by their mechanism of action. Antibiotics can also be classified by the organisms against which they are effective; and by the type of infection in which they are useful, which depends on the sensitivities of the organisms [4,5].

Increased use of antibiotics has caused the development of bacterial resistance to antibiotics. Antibiotic resistance can be defined as the ability of a microorganism to destroy or be unaffected by an antibiotic, or prevent it from entering the microorganism. Antibiotic resistance is a consequence of evolution via natural selection. The antibiotic action is an environmental pressure; those bacteria which have a mutation allowing them to survive will live on to reproduce. They will then pass this trait to their offspring, which will be a fully resistant population. Antibiotic resistance develops through mutation or plasmid exchange between bacteria of the same species. If a bacterium carries several resistance genes, it is called multi-resistance [5].

Several studies have demonstrated that the patterns of antibiotic usage greatly affect the number of resistant organisms which develops. Overuse of broad-spectrum antibiotics, such as second and third generation cephalosporins(*), greatly

(*) One of the common antibiotic's groups

hastens the development of methicillin[*] resistance, even in organisms that have never been exposed to the selective pressure of methicillin. Other factors contributing to resistance include incorrect diagnosis, unnecessary prescriptions, improper use of antibiotics by patients, and the use of antibiotics as livestock food additives for growth promotion.

Resistance to antibiotics is currently a major concern in treating infectious diseases. Antibiotics use and antibiotic resistance are clearly connected. Bacteria have developed mechanisms of resistance to all classes of antibiotics available for systemic use in human beings.

Resistance can be either inherent or acquired. Inherent resistance is a result of the normal genetic, structural, or physiologic state of a microorganism. This resistance is predictable and therefore recognized once the identity of the microorganism is known. Acquired resistance is when the organism has been able to either develop resistance by spontaneous mutation or has acquired a resistance mechanism from an external source. Acquired resistance can occur by acquiring resistance genes from other organisms.

Acquired bacterial resistance is common in isolates from healthy persons and from patients with community-acquired infections in developing countries, where the need for antibiotics is driven by the high incidence of infectious diseases [6]. Among isolates of commensal enteric pathogens, diarrhoea, and respiratory resistance is increasing, particularly to the first-line, inexpensive, broad-spectrum antibiotics. Furthermore, the introduction of newer drugs has been followed relatively quickly by the emergence and dissemination of resistant strains.

In general, antibiotic resistance results in gene action, bacteria acquire genes conferring resistance in any of three ways: (1) in spontaneous DNA mutation; (2) in a form of microbial sex called transformation, one bacterium may take up DNA from other bacterium; and (3) DNA acquired from a small circle of DNA called a plasmid, that can transfer from one type of bacterium to another. A single plasmid can encode different resistances to antibiotics [7,8].

Although bacterial antibiotic resistance is a natural phenomenon, the selection and spread of resistant organisms, which can often be traced to complex socio-

[*] Methicillin is another name (a proprietary name) of penicillin in penicillins group

economic and behavioural antecedents, contribute to the problem of worldwide antibiotic resistance.

1.3. The potential risk factors of antibiotic resistance

Antibiotic resistance has become a serious problem in both the developed and developing countries. One side effect of misusing antibiotics is the development of antibiotic resistance by the infecting organisms, similar to the development of pesticide resistance in insects.

1.3.1. Misuse of antibiotics by physicians in clinical practice

As inappropriate use increases the risk for selection and dissemination of antibiotic-resistant bacteria, one would expect that drugs more commonly affected by bacterial resistance in the developing countries are generally inexpensive with an acceptable price and popular broad-spectrum agents [9].

Unnecessary prescription of antibiotics seen in industrialized nations has also been documented in many developing countries, particularly in cases of acute infantile diarrhoea and viral respiratory infections. Clinical misuse of antibiotics may be more common among private practitioners who charge higher fees; the demand for antibiotics seen in private patients is higher and more drugs are available in private clinics than in public hospitals [10].

Health workers in many developing countries have almost no access to objective health information. Pharmaceutical company representatives typically outnumber practitioners and often adversely influence their prescription habits, as reflected by sales. Drugs labels and package inserts often fail to provide accurate information [11].

Antibiotic use in clinical practice alone cannot explain the high frequency of resistant organisms in developing countries. However, excessive clinical use is at least partially responsible for the escalating rates of resistance, especially in hospital settings.

1.3.2. Misuse of antibiotics by unskilled practitioners

Common forms of antibiotic misuse include taking an inappropriate antibiotic, in particular the use of antibiotic agents for viral infections like the common cold, and failure to take the entire prescribed course of the antibiotic, usually because

the patient feels better before the infecting organism is completely eradicated. In addition to treatment failure, these practices can result in antibiotic resistance.

In many developing countries, well-trained health personnel are scarce and cannot serve the entire population, especially in rural areas. Community health workers and others with minimal training treat minor ailments. The qualifications and training of community health workers, as well as the quality of care they provide, vary from county to country. Unskilled personnel are less aware of the deleterious effects of inappropriate antibiotic use [12].

1.3.3. Misuse of antibiotics by the public

In most of the developing countries, antibiotics can be purchased without prescription, even when the practice is illegal. In many African, Asian, and Latin American countries, antibiotics are readily available on demand from hospital, pharmacies, patent medicine stalls and hawkers. In rural Bangladesh, for example, 92% of drugs consumed for 1 month by more than 2,000 study participants came from local pharmacies; but only 8% of them had been prescribed by physicians [13]. People are encouraged to buy from unofficial distributors because drugs often are not available in government hospitals. Drug vendors usually have little or no knowledge of the required dosage regimen, indications or contraindications [14].

Antibiotic use in developing countries is underestimated. The quantity of drugs distributed within a country is calculated under the assumption that each person purchases a complete regimen. However, medication can be purchased in small quotas from roadside stalls, and distribution of locally produced or counterfeit antibiotics is not recorded. The motives for self-medication and antibiotics overuse by lay persons are similar to those for clinical abuse by health professionals; to cut costs and act expeditiously to treat confirmed or suspected bacterial infection. For example 50% to 80% of Bangladeshi patients infected with shigellosis admitted that they had taken at least one antibiotic in the 15 days before going to hospital [15].

Common cultural beliefs about antibiotics include the notions that there is a pill for every symptom; antibiotics are presumed to heal many diseases, including dyspepsia and headaches. Injections are seemed as more powerful

than pills. The misuse of antibiotics frequently becomes integrated into the local culture to prevent diarrhoea after eating suspected contaminated foods or to prevent sexually transmitted diseases [16].

Another cause of antibiotic abuse and selection for resistant bacteria is poor patient compliance. Firstly, physician-patient interactions are often inadequate. In Mexico, poor patient-physician communication was partially responsible for the non-compliance of patients with antibiotic regimens [17]. Secondly, because patients often travel long distances and incur large expenses for medical care, they are unlikely to return for follow-up visits. In addition, the patient may be unable to read medicine labels. Finally, because many drugs are expensive, indigent patients purchase incomplete regimens whenever possible and discontinue treatment when symptoms disappear but before the pathogen is eliminated [18].

1.3.4. Poor quality of antibiotics

Beside the risk for therapeutic failure, degradation products or adulterants in poor quality antibiotics can produce sub-inhibitory concentrations in vivo, which increase the selection of resistant strains. Drugs that do not comply with minimum standards are illegal in all countries. In many cases, therapeutic failure is the only indication of substandard drugs as laboratories to detect substandard drugs are uncommon, and when they exist, health workers, distributors and consumers are often unaware of them [19,20].

1.3.5. Dissemination of resistant organisms

- *Crowding and unhygienic conditions*

Residents of developing countries often carry antibiotic-resistant faecal commensal organisms [21,22]. Visitors to developing countries passively acquire antibiotic-resistant *Escherichia coli (E.coli)*, even if they are not taking prophylactic antibiotics; this suggests that they encountered a reservoir of antibiotics-resistant strains during travel. Several factors, such as urban migration with crowding and improper sewage disposal, encourage the exchange of antibiotic-resistant organisms between people and the exchange of resistance genes among bacteria, thereby increasing the prevalence of resistant strains [23].

Most residents of developing countries have no sanitary facilities for sewage disposal. Also pipe-borne water, often scarce in developing countries, is not always potable. The development of sanitation and other facilities is not always proportionate to the rapid rise in urban population. As urban migration continues, overcrowding increases and bad hygienic conditions are increasing the probability of the spread of antibiotic-resistant and commensal pathogens. Potable water disposal should reduce infections and the need for antibiotics and the subsequent development of antibiotic resistance [24].

Because tropical conditions encourage the survival of bacteria, more pathogens and commensal organisms are found in tropical environments than in temperate climates. The warm and humid tropical climate and the low levels of health care, hygiene and sanitation contribute to a relatively high prevalence of infectious disease in developing countries.

- *Inadequate hospital infection control*

Infection control practices in many hospitals in developing countries are rudimentary and often compromised by economic shortfalls and opposing traditional values. Due to improper disposal of hospital waste, the resistant organisms may be disseminated to the outside community. Untreated hospital waste in Uganda was often dumped into public sewers or thrown into rubbish heaps ravaged by scavengers [25].

1.3.6. Inadequate surveillance

- *Lack of sensitivity testing and surveillance*

Information from routine sensitivity testing of bacterial isolates and surveillance of antibiotics resistance, which provide information on resistance trends, including emerging antibiotic resistance, is essential for clinical practice and for rational policies against antibiotic resistance. The antibiotic susceptibility pattern of bacterial isolates in many developing countries is unknown, and testing cannot be done readily because equipment, personnel and consumables are scarce and expensive [26]. In most of the clinical infections, no clinical specimens are cultured. So if possible, a community-based antibiotic resistant surveillance data might be useful for medical doctors when they prescribed a medicine in the areas where they could not perform specific antibiotic-

susceptibility tests. For example, it was demonstrated that resistance among respiratory pathogens was infrequent in parts of Ethiopia. This information would help local Ethiopian health workers to treat such infections with inexpensive, broad-spectrum antibiotics [27].

National surveillance programs for antibiotics resistance, the norm in industrialized nations, are less common and less elaborate in developing countries. Current inferences about antibiotic resistance trends in developing countries are based on a small number of reports generated by handful of microbiology laboratories in urban areas - data not representative for a country, because wide variations in antibiotics resistance patterns may exist within countries [28].

- *Defective antibiotic susceptibility assay*

Well-standardized antibiotic susceptibility assays provide more reliable results. However, standard bacterial strains with which to assay new batches of antibiotics or antibiotic disks are not available in laboratory in many developing countries. Delayed transportation and breakdown of cold storage also affects the quality of antibiotics use as diagnostic reagents. Degraded antibiotic powders and antibiotic disks used for sensitivity testing might be lead to exaggerated estimates of bacteria resistant levels. Laboratory scientists in developing countries face difficulties in obtaining research supplies, which often require them to improvise by, for example, using injectable antibiotic formulations to measure MICs when standard antibiotic powders are not available. The report that clinical microbiologists in developing countries make their own disks from “local blotting papers” illustrates how improvisation can lead to inconsistent laboratory results and unreliable data [29].

1.3.7. Influence of antibiotics used in agriculture and aquaculture on antibiotic resistance of human bacterial pathogens.

The appearance of antibiotic resistance is directly linked with the use and overuse of antibiotics in human beings at home and at hospitals and in agriculture and aquaculture. It is causing concern worldwide that widespread use of antimicrobial agents in animal production may promote the development

of resistant bacteria or resistance genes that can be transferred to bacteria that cause disease in humans.

It has been estimated that in 1990; 300,000 kg of antimicrobial agents were used as growth promoters in animal feeds only in Netherlands [30]. 70% of total antibiotic production in the Unites States is use for the purpose of animal growth promotion [31]. In 1997, 10493 tonnes of antimicrobial agents were used in EU member states and Switzerland, with 3494 tonnes being used in veterinary medicine [32].

Most antibiotic use is in two areas: in humans in the community, and in animals for growth promotion and prophylaxis. The data in the table suggest that up to 75% of antibiotic use is of questionable therapeutic value [33].

Table 1: *Frequency of antibiotic use*

Antibiotics are used	Types of use	Questionable use
Human use (50%)	20% Hospital 80% Community	20-50%
Agricultural use (50%)	20% Therapeutic 80% Prophylactic /growth promotion	40-80%

Antibiotics are used in food-producing of animals for three major reasons. First, antibiotics are used to treat sick animals. Second, antibiotics are used in the absence of disease to prevent diseases during times when animals may be susceptible to infections. This use affects a larger number of animals, which increases the likelihood of selecting for organisms that are resistant to the antibiotic. Third, antibiotics are commonly given in the feed at low doses for long periods to promote the growth of animals. All antibiotics in the environment will increase the pressure for bacteria to become resistant [34,35].

Resistant bacteria may be transferred to humans though the food supply or direct contact with animals (***figure 1***). For example, *Campylobacter* lives in the intestines of chicken. In 1989, none of the *Campylobacter* strains from ill persons that CDC (Atlanta) tested were resistant to fluoroquinolone. But in 1995, the FDA (Food and Drug Administration) approved the use of

fluoroquinolones in poultry. Soon after that, doctors found that *Campylobacter* from ill persons were resistant to fluoroquinolone [36].

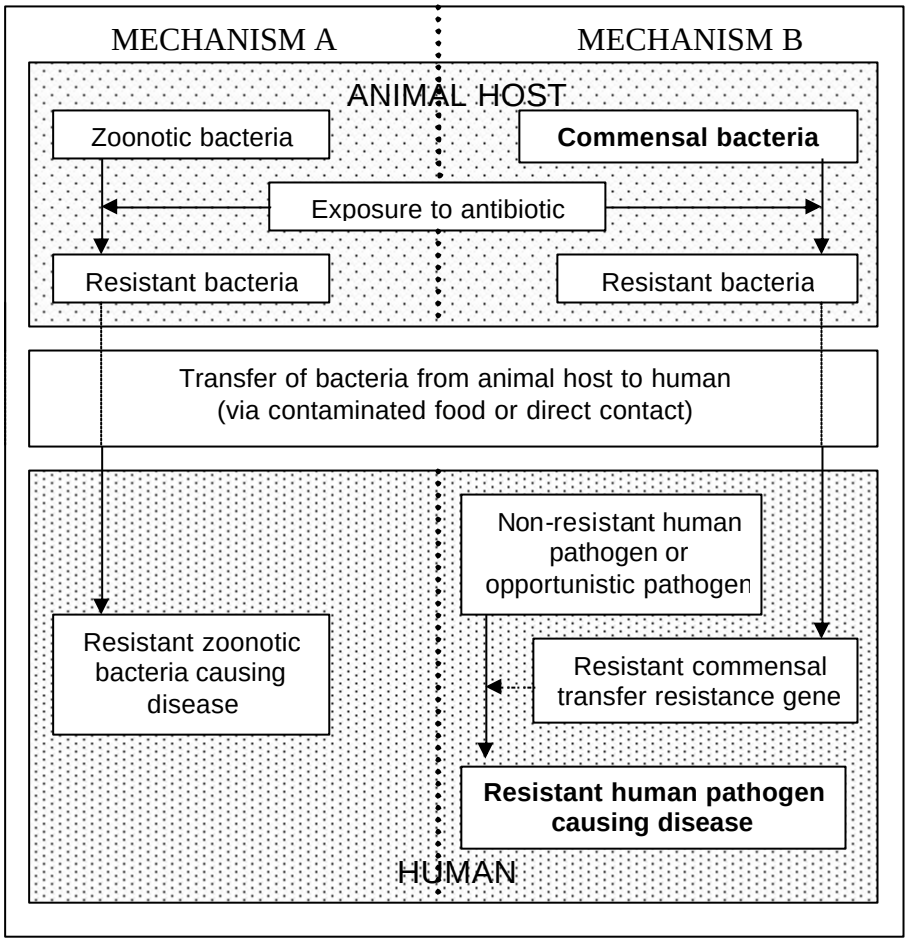

Figure 1: *Spread of antibiotic resistance from animals to humans.*

There are many potential mechanisms by which antimicrobial resistance can have adverse effects on human health:

- A mechanism in which the individuals become infected only because they are taking an antimicrobial agent (for unrelated reasons) to which the pathogen is resistant [37].
- A mechanism involving the linkage of virulence traits to resistance traits so that resistant organisms may be more virulent than susceptible organisms [37].
- A mechanism that renders treatment ineffective by the choice of a drug to which the pathogens is resistant or may be complicated by the need to use an agent with less desirable attributes than would otherwise be the case [37].

- A mechanism that is the animal equivalent of the attributable fraction: resistant pathogens acquired by animals in food may be transmitted through the food chain to humans [37].
- The commensal flora of animals can acquire resistance traits; from this reservoir, resistance traits could find their way through the food chain to commensal and pathogens of humans [37].

The problem of the development of antibiotic resistance in bacteria has been subject to specially commissioned reports under the auspices of a variety of governmental and international bodies. Much of the focus of these reports has been upon the use of antibiotics in domestic animals and, in particular, their use as feed additives for the purpose of growth promotion.

For example, the network in Spain covers the three critical points of veterinary responsibility, bacteria from sick animals, bacteria from healthy animals and bacteria from food animals. Surveillance of sick animals was first implemented using *E.coli* as the sentinel bacterium. Surveillance of *E.coli* and *Enterococcus faecium* from healthy pigs was implemented in 1998. In July 1999, data collection on *Salmonella* spp. was initiated in poultry slaughterhouses. Additionally, the prevalence of vancomycin(*) resistant *E.faecium* was also monitored. This network has specific topics of interest related to methods of determining resistance, analysis and reporting of data, methods of use for veterinary practitioners and collaboration with public health authorities [38].

1.3.8. Other influencing factors

Lack of resources hampers implementation of most strategies against antibiotic resistance. Statistics from the World Bank shown that the developing countries spent 41 USD per person on health in 1990, compared with 1,500 USD per person spent by the industrialized countries [39]. Disease prevalence as measured by disability adjusted life years and by communicable disease in particular is much greater in developing countries. As the result of such gross under-funding, the drug supply is chronically inadequate or at best erratic in health facilities in many countries.

(*) Vancomicin is a useful for treating methicillin resistant *Staphilococcus aureus*

The conflicts have recently led to a breakdown in health services and sanitation and rapid dissemination of resistant pathogen, particularly in Sub-Sahara Africa and Asia.

Even in developing countries not at war, political corruption and mismanagement of funds, personnel, and development programmes have created large populations living in miserable poverty and at high risk for infectious diseases [29]. Medical expenses, days lost from work, and transportation cost account for substantial economic loss. The cost of medical treatment, even subsidized treatment, is beyond the means of many patients. Poorly paid health workers sometimes illegally extract fees from patients [29]. Thus persons with some communicable diseases, unable to afford medical treatment, may infect others. Poverty also interferes with patient compliance, which in turn promotes the emergence of antibiotic resistance during short-term therapy of acute infections and long-term therapy of chronic infection, such as tuberculosis [40].

1.4. Country profile

1.4.1. Geography

Viet Nam is located in the centre of Southeast Asia. The country lies in the eastern part of the Indochina Peninsula, bordered with China to the north, Laos and Cambodia to the west, and the East Sea and Pacific Ocean to the Southeast. The country has a land area of 329,560 square kilometres and it is divided into four main regions following the geographical nature. These are Northern, Central, Southern and Highland [41].

Viet Nam is located in both a tropical and a temperate zone. It is characterised by strong monsoon influences, but has a considerable amount of sun, and a high rate of rainfall and humidity. The annual average temperature ranges from $22^{O}C$ to $27^{O}C$. The rainfall is generally abundant over the entire country ranging from an average of 2,000 mm in the north to 1,700 mm in the south with a small pocket of 700 mm in Thuan Hai province. There are two distinguishable seasons in the whole country, the cold season occurs from November to April and the hot season occurs from May to October. The difference in temperature between the two seasons of the provinces in Southern

Viet Nam is almost unnoticeable, averaging at 3^{O}C. The most noticeable variations are found in the Northern provinces where differences of 12^{O}C are observed. There are essentially four distinct seasons most evident in the Northern provinces. In Ha Noi, the average temperature is 23^{O}C. In Ho Chi Minh City it is 26^{O}C [42].

***Figure 2**: The map of Viet Nam* [42]

1.4.2. Administration

Administratively, Viet Nam has divided into 59 provinces and 5 municipalities, which are relatively self-supporting. The districts are subclassions of the provinces; and communes are the smallest administrative units [41]. Each level (national, province, district and commune) has a people's committee and people's council, which are represented for government and people.

1.4.3. Population and demographic characteristic

According to the last national census from 1999, 79.6 million inhabitants are living in Viet Nam, but following the statistical record in 2003, there were over 81 million people with population growth rate around 1.29 %. Until July 2004, an estimate of population of Viet Nam is 82,689,518 people. Median age of males is 24 year, and females is 25.9 year. In the total population, the sex ratio is 0.98 male / female, and life expectancy is 70.35 years. Birth and death rates are 19.58 births and 6.14 deaths per 1000 population. Total fertility rate is 2.22 children born / woman [41].

There are 56 ethnic groups; among them 85% - 90% belong to the Kinh ethnic group. Other groups are distributed in the mountainous and forest areas from north to south. Around 20% of the population live in urban areas [41].

1.4.4. Economy

Viet Nam is a poor, densely-populated country that has had to recover from the ravages of war. Substantial progress was achieved from 1986 to 1996 in moving forward from an extremely low starting point - growth averaged around 9% per year from 1993 to 1997. In the total GDP, agriculture contributed 21.8%; industry contributed 40.1%; and services contributed 38.1%. In Viet Nam, the population below the poverty line was around 28.9% (estimated in 2002) [41]

1.4.5. Health sector

The health care system in Viet Nam is organized along a four-tiered pyramid. At the top of the pyramid is the Ministry of Health, which is the main national authority in the health sector and together with the provincial, district and commune People's Committees, formulates and executes the health policy and programs in the country [43].

At the central level, the Ministry of Health (MOH) is a national authority responsible for health care in the whole country. It also directly controls the activities of central medical and pharmaceutical training institutions, academic institutions, and national hospitals and national research institutes. The country now is divided into 64 provinces and municipalities, which are relatively self-supporting. Each province has a health bureau, serving an average population of 1.2 million, directly coordinate by MOH as well as the provincial committees.

In each province, there is at least one general hospital, and may be have some specialized hospitals, clinics and pharmacy stores.

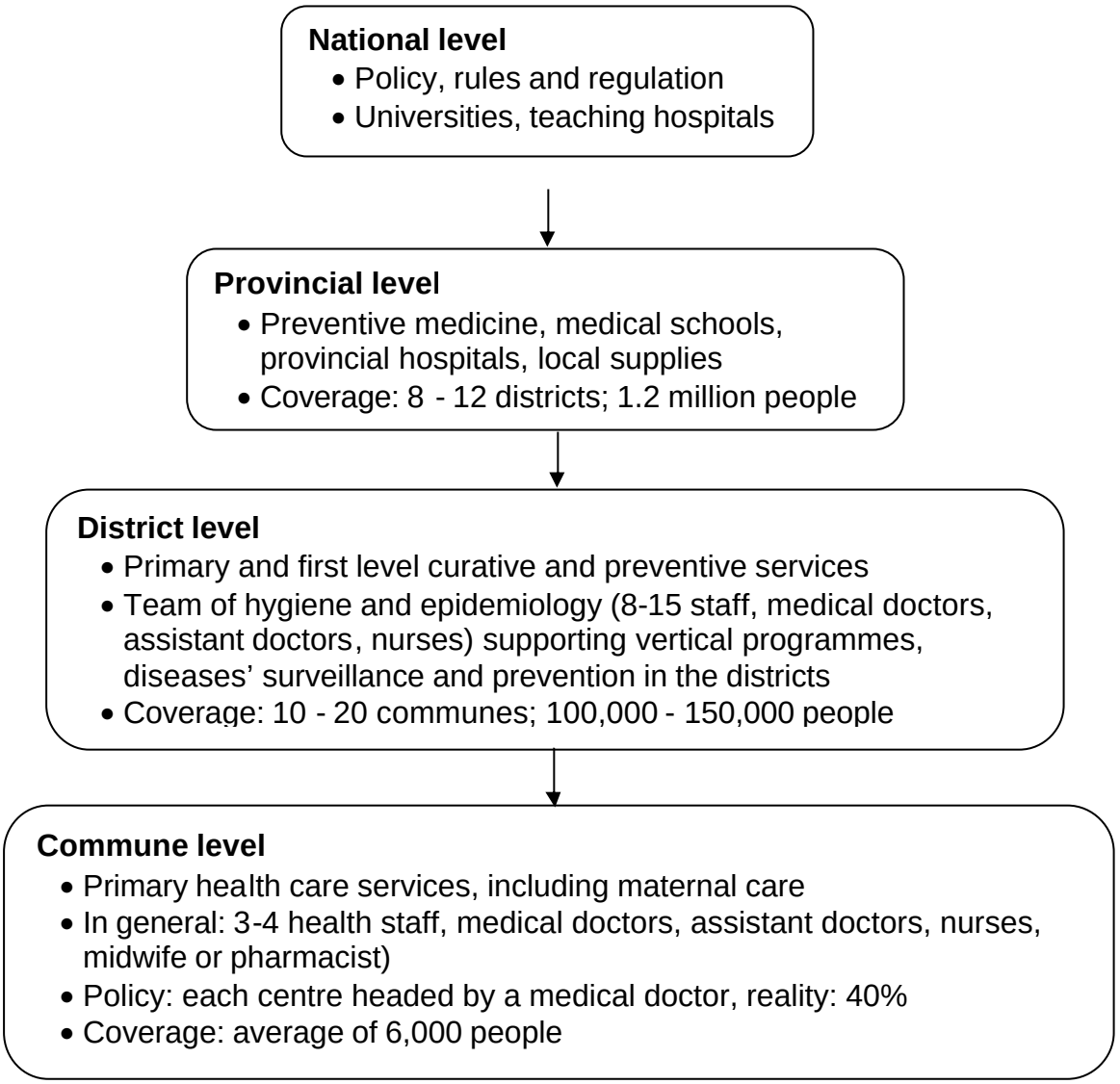

Figure 3: *Health system in Viet Nam* [43]

A province is divided into districts. In this level there is health centre with public pharmacy and a district hospital serving a population about 100,000 to 150,000 persons in each district. The last level is community level with a community health centre. Each community health centre has 3 to 4 health staffs responsible for providing primary health care and implementing preventive health programmes, included mother and child health, family planning, immunization programmes. Each community health centre is coverage an average 6,000 inhabitant. Viet Nam has a lot of private clinics and pharmacies,

authorised by the ministry of health, especially in the big cities such as Hanoi, Ho Chi Minh City, etc. and in the towns of the provinces [44].

Despite being one of the poor countries in Asia, Viet Nam's overall state of health, as measured by conventional indicators, is much better than would be expected for a country at its level of income per capita. There is no question that Viet Nam's achievements in reducing infant mortality are impressive and have few parallels in the world. The infant mortality rate of Viet Nam is already among those with the lowest rate in the Asia-pacific region [43,44].

***Table 2**: Infant mortality rate of some countries in the Asia-pacific region (by the year 2003)* [41]

Country	**Infant mortality rate (per 1000)**
Laos	88.94
Cambodia	75.94
Burma (Myanmar)	70.35
India	59.59
East Timor	47.41
Indonesia	38.09
Viet Nam	**30.83**
Philippines	24.98
Thailand	21.83
Malaysia	19.00
Singapore	2.29

Viet Nam has experienced an epidemiological transition during the last 20 years with a major decline in the share of communicable diseases in mortality and morbidity. From 1976 to 1997, while communicable diseases accounted for 50-56% of mortality and morbidity cases in 1976, the corresponding figure was 27% by 1997. These shifts reflect the success of communicable disease control programs especially the expanded program of immunization, which has dramatically reduced the incidence of vaccine-preventable diseases in Viet Nam

Despite the decline in their incidence, infectious diseases continue to remain a major public health problem in Viet Nam. In 2002, for instance, malaria, diarrhoea, and respiratory tuberculosis were among the ten leading cause of admissions at public hospitals together accounting for nearly 600,000 cases [45].

1.4.6. Drug policy

Since the mid 1970s the Swedish government, through SIDA (Swedish International Development Cooperation Agency), has financed a development programme within the health sector in Viet Nam. Drug policy and control is one of six areas for support in the present five years' plan, which started in October 1994. The Ministry of Health in Viet Nam has the overall responsibility for the Viet Nam - Sweden Health Cooperation. Recently Viet Nam has developed a National Drug Policy. Increasing efforts are being made to improve pharmaceutical sector performance in low and middle-income countries. An essential tool for such work is an objective and standard method of assessment, which can be used to promote evidenced-based National Drug Policy development and implementation. The average drug expenditure per capita has steadily increased in Viet Nam, and at the time of this study a National Drug Policy was being developed. However, one study assessed the Vietnamese pharmaceutical sector 1991-1994, focusing on the standard of the drug quality control system, availability of drugs and rational use of essential drugs in the private and public sectors by means of standardised indicators. This study indicated that the quality control system is impaired and does not have capacity to quality control all drugs on the market. The availability of essential drugs is good, whereas essential drugs are poorly prescribing. Injections are commonly used, and there are a high average number of drugs per prescription, both in the public and private sectors. Violations are common and enforcement of regulations weak. On top of this, there is an active commercial advertising and marketing of drugs [46].

1.4.7. Consumption of antibiotics

In Viet Nam, the same as in most developing countries, antibiotics can be bought in many places from big cities to remote areas, and there is little regulation of their use. Self-medication is a common behaviour of the

Vietnamese population. Reportedly 40 - 60% of Vietnamese people depend on self-medication. Mothers use antibiotics as if such drug were panaceas. There are insufficient public health education, no control over pharmaceutical promotion, and no efficient drug policy and regulation [47,48]. The inappropriate use of antibiotics, both regarding the choice of drugs and their dosage, is frequent in the communities and in hospitals. Self-medication has been associated, in particular, with the overuse and inappropriate use of antibiotic drugs, and this has caused antibiotic resistance levels in Viet Nam to reach epidemic levels.

Laboratory investigation work in microbiology concerning identification and antibiotic sensitivity of bacteria is not well standardised, especially in the rural areas far from big medical centres, and may be misleading for the estimation of the prevalence of antibiotic resistance among different bacteria. There is lack of facilities to process microbiological specimens in primary health care. Bacteriological findings in hospital laboratories do not always reflect the primary cause of neither morbidity nor the sensitivity to antibiotics at primary health care level, as patients have often been subject to one or more antibiotic treatment courses before referral.

1.3.8. The prevalence of enterobacterial resistance to antibiotics in Viet Nam

Antibiotic resistance is rising rapidly in many bacterial pathogens in Viet Nam. Recent experiences with multiresistant *Salmonella typhi* (*S.typhi*), *Echerichia coli* (*E.coli*), and *Streptococcus pneumoniae* illustrate the emerging problems of antibiotics resistance in community-acquired pathogens that are typical of many countries.

- *Salmonella spp:*

In Viet Nam, the resistance to antimicrobial agents is rather low in *Salmonella*: 10% to tetracycline, 9.5% to chloramphenicol, and no cefotaxime, ciprofloxacin and trimethoprim / sulfamethoxazole - resistant isolates were found[49].

However, high level of multidrug-resistant (MDR) of *S.typhi* have been reported at different locations in Viet Nam. The proportion of MDR isolates increase from 27% to 89% in 1995 [50,51]. In Southern Viet Nam, several studies

have shown that *S.typhi* became resistant to second-generation quinolones, which are the drugs of choice (DOC) for treatment of typhoid fever in Viet Nam. The MDR strains are an increasing burden for public health interventions and clinical treatment of this disease.

Table 3: *Resistance (%) of Salmonella spp. to antimicrobials in Viet Nam* [49]

Bacteria	AMP	AMS	CFM	NAL	CIP	CHL	TET	TSX
S.enteritidis	2.7	2.7	0	2.7	0	6.7	4	0
Salmonella spp.	6.3	6.3	0	3.2	0	9.5	10.5	0

Nevertheless, a recently study in Son Lla province found that *Salmonella typhi* was sensitive against with the first line antibiotics in the communities [52]. These findings were different from other studies in Viet Nam [51,53]. The sensitivity of *S.typhi* strains to first line antibiotics might be due to the restricted usage of these drugs in Son La, a province of Northern Viet Nam. MDR *S.typhi* strains carried a 140-kb R-plasmid that encodes MDR. This R-plasmid can transfer from mother to daughter cells. Sensitivity to the first line antibiotics in these studies may be related to the loss of this R-plasmid [51,53].

- <u>*Shigella spp.:*</u>

Antibiotic susceptibility was determined in *Shigella* spp. strains isolated in Viet Nam during 1998-2003. Almost all *Shigella flexneri* strains collected were resistant to trimethoprim/sulfamethoxazole (98%), chloramphenicol (98%), tetracycline (98%), ampicillin/sulbactam (95%), and ampicillin (95%). Whereas *Shigella sonnei* obtained during the same period were relatively less resistant to ampicillin (7%) with similar resistance rates to other antibiotics tested: trimethoprim / sulfamethoxazole - 100%, chloramphenicol - 72%, tetracycline - 93%. All *Shigella* isolates were susceptible to nalidixic acid and 4-5% of *Shigella* were resistant to ciprofloxacin [49].

- <u>*Enterococcus spp:*</u>

The *Enterococcus* antibiotic susceptibility in Viet Nam (1995 - 2003) revealed that 84% and 85% of *E.faecalis* isolates were susceptible to ampicillin and ampicillin/sulbactam respectively, the frequency of high-level resistance

amounted to 44% for streptomycin and 25% for gentamicin. All strains were susceptible to vancomycin, 75% to ciprofloxacin. As for *E.faecium* and rare species of *Enterococcus* spp. more than 70% of them were resistant to ampicillin and ampicillin/sulbactam, high - level resistance to aminoglycosides exceeded 60% [49].

- <u>*Escherichia coli*</u>

  E.coli is the most common causative agent of diarrhoeal disease. The species may be classified according to their mechanism of pathogenicity as follows:

  + Enterotoxigenic *E.coli* (ETEC), which are agents of cholera like diarrhoea, frequent in tropical areas, lacking proper hygiene, and which affect small children under 5 year of age especially, and travellers to these countries. In those regions, which have high standards of hygiene, ETEC are rare, although they may sometimes be the cause of epidemic diarrhoea in day-care nurseries. The diagnosis of these microorganisms may be affirmed by demonstrating the presence of the heat-stable enterotoxin (ST). Like *Vibrio cholerae*, they attach to cells of the intestines but do not penetrate them [54,55].

  + Enteropathogenic *E.coli* (EPEC): EPEC is an important category diarrheagenic *E.coli* that has been liked to infant diarrhoea in the developing countries. EPEC induce watery diarrhoea similar to ETEC, but they do not possess the same colonization factors and do not produce ST (heat-stable) or LT (heat-labile) toxin. Once defined solely on the basis of O and H serotypes; EPEC is now defined on the basis of pathogeneticity characteristics [54,55].

  + Enteroinvasive *E.coli* (EIEC) is the cause of dysenteric syndromes. Epidemiologic studies of EIEC mostly describe outbreaks. In sporadic cases, many EIEC strains are probably misidentified as *Shigella* Spp or non-pathogenic *E.coli* strains. EIEC outbreaks are usually food-borne or waterborne, although person-to-person transmission does occur [54,55].

  + Enterohemorrhagic *E.coli* (EHEC): EHEC are represented by a single strain (serotype O157:H7) which causes a diarrhoeal syndrome distinct from EIEC (and *Shigella*) in that there is copious bloody discharge and no fever [54,55].

+ Enteroaggregative *E.coli* (EAggEC): These strains are associated with persistent diarrhoea in young children. The distinguishing feature of EAggEC strains is their ability to attach to tissue culture cell in an aggregative manner[54,55].

According to the data from NIHE in 2003 for the antibiotic resistance of pathogenic bacteria, the highest frequency of resistance was observed to ampicillin (33.3%) and trimethoprim / sulfamethoxazole (18.4%). While fluoroquinolones (norfloxacin and ciprofloxacin) proved to be the most active antimicrobials tested [49].

EPEC is one of the most frequent causes of diarrhoeal disease, especially in children under 5 years of age in developing countries. Uncontrolled use of antibiotics in the community might lead to antibiotic resistance. Resistant *E.coli* strains efficiently exchange genetic material with the other enteric bacteria and spread resistance genes. It may make other bacteria acquires genes conferring resistance and become antibiotic resistance.

1.5. Province profile

1.5.1. Study place:

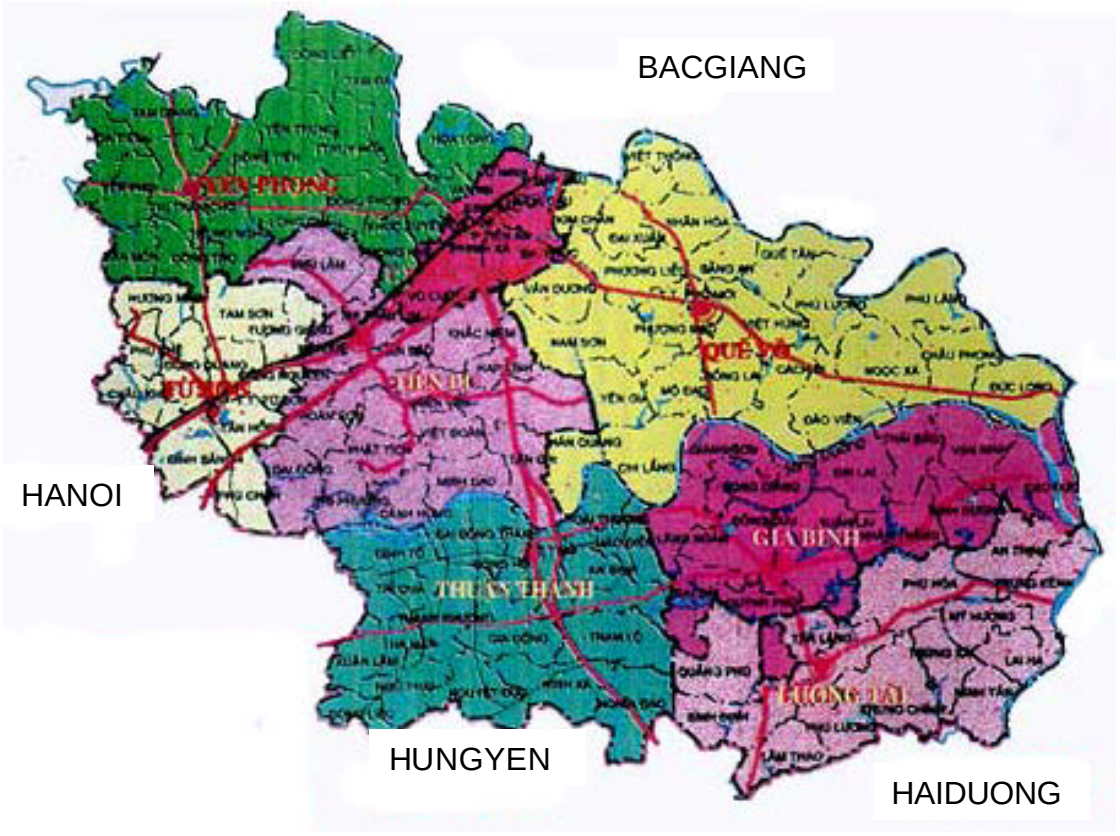

Figure 4: *Bac Ninh administration map* [56]

Bac Ninh is a province of Northern Viet Nam, serving as the northern gate of Ha Noi. The province is 803.87 square kilometres for a population of 984,121 inhabitants equal 1.2% of the total population of Viet Nam (by the year 2003), 620,944 persons are over the age of 15. The Bac Ninh province is located next to Noi Bai International Airport and inside the triangle of key economic regions: Ha Noi, Hai Phong, and Quang Ninh. It has important communication axes that link the locality with economic, cultural and trading centres in the North such as National Highway 1A, Hanoi - Lang Son Highway, National Highway number 18, National Highway number 38, and the trains - Viet Nam Railroad that spans Viet Nam and China [57].

The economic growth rate of Bac Ninh province in 1997 was 10.2%; in 2000 was 16.6%; and in 2001 was 14.1% [57].

Particularly, after being upgraded, National Highway number 18 will become a convenient communication line, which reaches Noi Bai International Airport and Cai Lan deep - water seaport (Quang Ninh province). Besides, Bac Ninh has a convenient river route that links the province with neighbouring Haiphong seaport and large economic centres in the North. The province now is divided into 8 districts (Yen Phong, Tien Du, Tu Son, Que Vo, Thuan Thanh, Gia Binh, Luong Tai and Bac Ninh town).

Bac Ninh has humid tropical climate zone with a cold winter. The annual average temperature is 23.3^{O}C and average humidity is 79%. The average temperature in the hottest month is 28.9^{O}C (July) and lowest month is 15.8^{O}C (January). The average rainfall annually is ranging from 1,400 mm to 1,600 mm but it is not equilaterally distributed in whole year. From May to July, the rainfall is 80% of the year [57].

1.5.2. Health sector:

There are 147 health facilities and clinics to examine and treat (included two hospitals) in Bac Ninh province. Bac Ninh hospital is a general provincial hospital with 560 beds, offering health care mostly to people in Bac Ninh. It is the reference hospital for the province with national standard. Annually, approximate 15,000 patients are admitted to the Bac Ninh hospital, some of them came from contiguous areas [57].

There is a district general hospital in each district, including a laboratory and a post for hygiene, epidemiology and malariology. The whole province has 125 communities; each commune has a community health centre responsible for providing primary health care and implementing preventive health programmes.

Table 4: *The prevalence of some enteric infectious diseases in Bac Ninh Province.* **(Statistics record 2003 - NIHE)**

	Diseases	**Morbidity / 100,000**	**Mortality / 100,000**
1	Cholera	0	0
2	Typhoid	0.91	0
3	Shigelosis	15.65	0
4	Shigelloid syndrom	351.80	0
5	Diarrhoea	1098.34	0

According to the statistical record of NIHE in 2003, the prevalence of diarrhoeal diseases in Bac Ninh was seemed in high level, [49].

Diarrhoea cause by mulltidrug-resistant bacteria has been recognized as an important public health problem and is a research priority of the diarrhoeal disease control program of the World Health Organization. Among of these bacteria, strains of the different diarrhoeagenic categories of *E.coli* are among the most important causes of acute enteritis and subsequent morbidity and mortality in children in developing countries. Knowledge of local antibiotic therapy patterns, to determine risk factor contributing to antibiotics resistance of the bacteria and to investigate mechanisms of resistance in Viet Nam are important in selecting the appropriate therapy, and may help to create a basis for the promotion of policy of prudent use of antibiotic. A basis for continuous surveillance of antibiotic resistance may also be laid.

Table 5: *Comparison of morbidity of enteric diseases in some provinces in North-Plain 2003 (Information from EPI of Viet Nam)*

	Province	Morbidity / 100,000			
		Typhoid	Shigelosis	Shigelloid synd.	Diarrhoea
1	Ha Noi	1.41	0.00	127.34	1555.71
2	Hai Phong	1.43	22.52	153.23	318.40
3	Thai Binh	0.05	3.51	135.03	4675.65
4	Nam Dinh	0.00	0.00	155.17	627.73
5	Ha Nam	0.00	2.33	577.75	1863.83
6	Ninh Binh	8.94	5.63	253.94	1208.03
7	Bac Giang	0.51	6.69	347.04	1057.70
8	Bac Ninh	0.91	15.65	352.80	1098.34
9	Phu Tho	0.00	11.76	271.81	712.43
10	Vinh Phuc	0.09	0.53	306.46	821.70
11	Hai Duong	0.29	5.68	319.79	897.55
12	Hung Yen	1.79	11.92	677.64	1246.66

CHAPTER II: RESEARCH QUESTION AND OBJECTIVES

2.1. Research question

What are the risk factors associated to antibiotic resistance of bacteria from diarrhoeal patients and what is its prevalence in Bac Ninh hospital, a province hospital of Northern Viet Nam?

2.2. Research objectives

- To describe the antibiotic susceptibility of EPEC strains isolated fom the diarrhoea patients admitted to Bac Ninh hospital.
- To identify the risk factors that contribute to antibiotic resistance of bacteria from diarrhoeal patients who were admitted to Bac Ninh hospital in Bac Ninh province, Northern Viet Nam in 2004.
- To study the pattern of antibiotic use for diarrhoeal patients in the community.

CHAPTER III: METHODS AND MATERIALS

3.1. Study design and sample selection

3.1.1. Selection of study area

Bac Ninh Province was chosen for the study because it is not so far from Ha Noi. The inhabitants have many opportunities to buy antibiotics in many places (in the local areas and in Ha Noi city). In addition, following the statistical record of NIHE in 2003, the prevalence of enteric infectious diseases in Bac Ninh province was at a high level, especially concerning diarrhoeal disease [49].

3.1.2. Study design

In the epidemiological approach to investigate the cause of a disease, a case-control study, across-sectional study or a cohort study may be used [58,59,60]. A cross sectional study measures the prevalence of health outcome in a population at a point in time or over a short period, or both. The cross sectional study analyses data collected on a group of subjects. This information can be used to explore aetiology [59,60,61]. Cross-sectional study is a type of observational descriptive investigation, in which exposure and non-exposure statuses are assessed simultaneously among individuals in a defined of population. Its result can provide and help the public health administrators in assessing the health status and health care needs of a population [62].

In this study, a hospital-based cross sectional design has been used with both quantitative and qualitative methodology. All diarrhoeal patients admitted to Bac Ninh hospital from August 2004 to December 2004 were included for the study as suspected cases. A stool specimen would be collected immediately after admission and examined for etiological agents. The cases were be defined by a positive stool culture of EPEC from diarrhoeal patient.

The advantage of a cross-sectional study is that all the information is collected at the same time, and subjects are contacted once when convenient for them. It also helps to assess variety in the phenomenon under investigation at one specific time [63]. In one special circumstance, a cross-sectional study can be considered as a type of analytic study and used to test epidemiologic hypotheses [63,64].

One disadvantage of cross-sectional surveys is that cannot assure without further evidence that the sample represents a broader universe. These studies only represent those individuals who participated in the study. Thus the method of drawing the sample and sample size is critical to the accuracy of the study and its potential for generalizing [65].

There was not previous study about antibiotic resistance in Bac Ninh province; therefore a cross-sectional design is also suitable for getting base line data in the study area.

Data were collected using both microbiologic and epidemiologic methods. All the EPEC strains, which were isolated from the patients, were tested by microbiological methods to find the susceptibility profile of the strains to identify the genes encoding antibiotic resistance. The questionnaires mapped demographics and socio-economic variables, what type of antibiotic was used, how long treatment lasted, where the antibiotics had been purchased and what type of treatment information the patient had retained, etc. The questionnaire contained both pre-designed answer options and space for open answers.

3.1.3. Study population

- *Identify suspect cases:*

  Patients, who had acute diarrhoea (defined as three or more diarrhoeal movements per day) admitted to Bac Ninh provincial hospital from August to December 2004, were asked to participate in the study as suspect cases. Physicians performed a physical examination, checked clinical signs and prepared for treatment. An interview should be carried out after the status of the patient was improved [27,66]. A stool specimen waas collected immediately after admission, before treatment, and used for etiological studies.

- *Participant selection:*

  A participant was defined as a diarrhoeal patient with an EPEC strain, isolated from his/or her stool specimen, from July to December 2004 in Bac Ninh hospital. The antibiotic sensitivity tests were carried out in their EPEC strains to define exposure and non-exposure cases.

For more study, all of the EPEC strains should be tested by molecular biology technique (PCR technique) to detect antibiotic resistant genes [67,68].

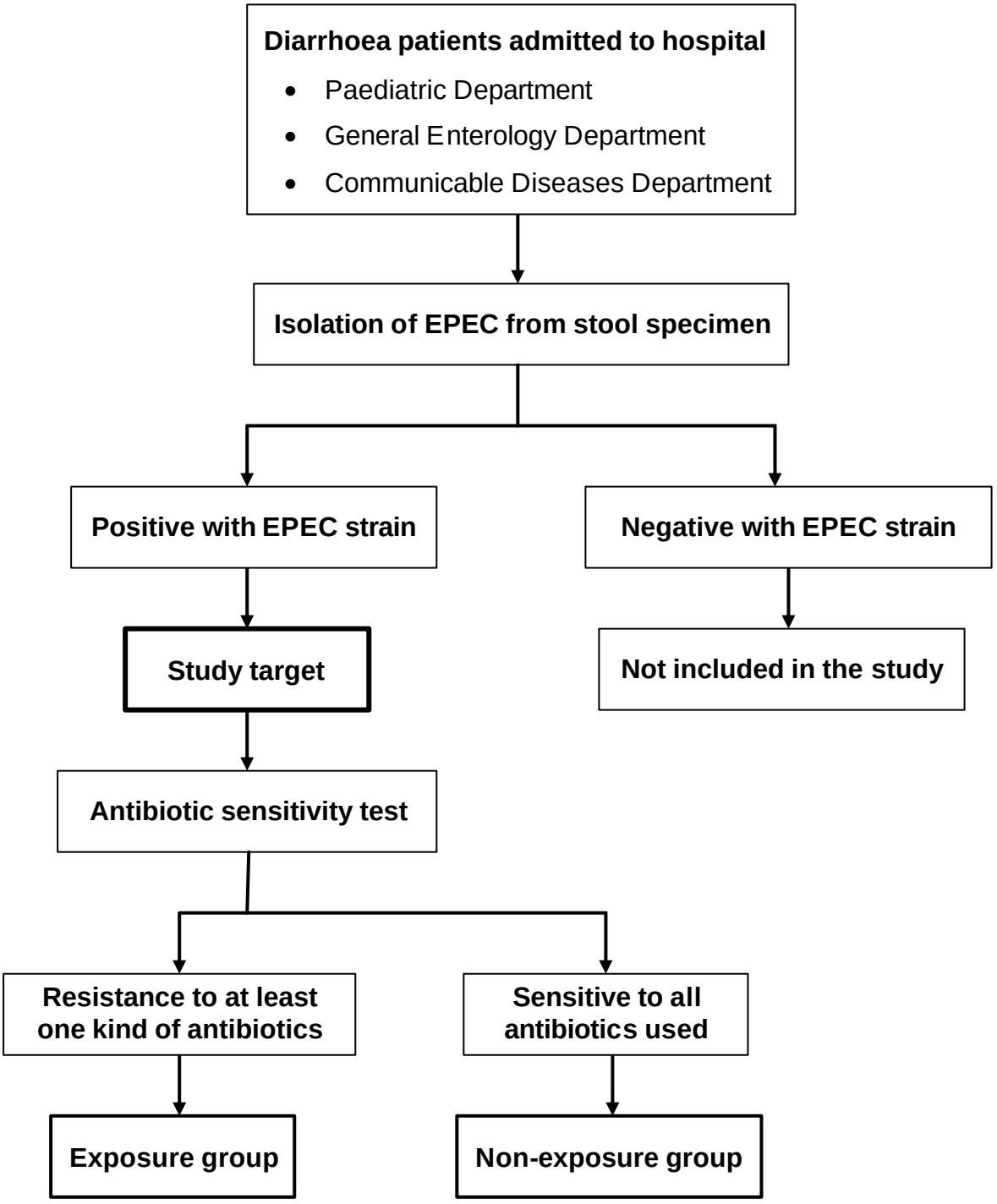

***Figure 5**: Decision of flow chart for identification of study target*

4.1.4. Sample size

Sample size calculation: we used Statcalc in Epi-info 6.0 for calculation of the sample size. We have no basis for the assumptions and the sample size must rather be based on convenience sampling. For the calculation of the sample size, a confidence interval of 95%; a power of 80% (1 - ß); unexposed/exposed = 1/1; and an expected relative risk of 0.95 was chosen:

236 should have been included according to the sample size calculation. However, because of some limitations during the study, we have just collected a total of 227 cases positive with EPEC from 526 diarrhoeal patients admitted during the study's period. This allowed us to calculate the prevalence of drug resistance of EPEC strains to the most frequently used antibiotics in Bac Ninh province.

3.1.5. Inclusion criteria

- Diarrhoeal patients cause by EPEC strain admitted to Bac Ninh hospital from August to December, 2004.
- The strains have been collected from the diarrhoeal patients admitted before treatment with antibiotic drug.

3.1.6. Exclusion criteria

- Any diarrhoeal patients who declined to participate in the study either before or during the interview process
- Diarrhoeal patients caused by other reasons, such as viruses, food poisoning, etc. who were admitted to Bac Ninh hospital from July to December, 2004.

3.2. Research teams

The study was conducted in collaboration with researchers in Department of Bacteriology at the National Institute of Hygiene and Epidemiology (NIHE), staffs of three clinic departments and Microbiology Department in Bac Ninh hospital. All their equipments and resources available were used in this study.

3.2.1. Department of Bacteriology - NIHE

Responsibilities:

- Training interviewers,
- Training skills for collecting sample,
- Training staffs working in the laboratory of Bac Ninh hospital for the study,
- Regular checks of quality on skill interview,
- Participate interviews with suspect cases if it possible

- Organize the meeting or seminar for reporting progress, discussion of problems, maintaining good morale,
- Reconfirmation of enterobacteriacea strains,
- Carrying out molecular biology techniques,
- Collecting and analysing the data.

3.2.2. Clinical departments in Bac Ninh hospital:

Responsibilities:

- Interview and collect the information of suspect cases through questionnaires,
- Collect stool samples of diarrhoeal patients who admitted hospitals.

3.2.3. Microbiology department in Bac Ninh hospital

- Collecting stool specimens which had been taken in clinical departments,
- Isolation and specify EPEC strains from stool samples,
- Collecting questionnaires had been interviewed from three clinical departments,
- Transportation EPEC strains and questionnaires collected to NIHE

3.3. Data collection

In this study, both quantitative and qualitative methods were used. Stool samples from diarrhoeal patients were collected for culture to detect EPEC. The information was collected through semi-structured interviews regarding risk factors of antibiotic resistance; collecting information and observation the fact situation of infectious control in hospital, sewage system and improper disposal of hospital waste in Bac Ninh hospital.

3.3.1. Training of research assistances and pre-testing

- *Pilot study*

The pilot study was conducted with the first ten suspect cases with both a collect stool sample and an interview according to the questionnaire. After the pilot study, a seminar was organised for researchers and interviewers to learn from the experience of interview skill, to collect in sampling and laboratory skill.

All encountered problems were discussed and solved. Some adjustments based on fact of interview progress were made in the questionnaire to make it more suitable. For example, we changed the sequence of answers in the question "Where do you go when you are getting sick?"; move out the question about the place of antibiotic keeping; added the appropriate answering in the question number 40; ect.

- *Training skill of interviewers*

Eight interviewers were selected, two of them from the enteric pathogens laboratory at NIHE and six medical doctors from the Bac Ninh hospital; who had responsibility for each department in the study place (included Paediatric Department, General Enterology Department, and Communicable Diseases Department). In this session, the interviewers were instructed how to ask the questions and how to report exactly what the respondent answered. The interviewers had to practise together to ensure a standardised way of collecting information.

- *Training for laboratory skills*

Training of laboratory skills was an important step in this study. We spent three days for training of the technicians in order to improve their skill to identify *Enterobacteriaceae* following a standard protocol from NIHE.

3.3.2. Epidemiology method:

Data were collected through semi-structured interviews with a questionnaire. Face to face interviews were conducted in Vietnamese. Some of the questions were designed as multiple choices, and some of them were open questions. Patients admitted with acute diarrhoea (or guarantors for patients under 5 years of age) were asked to give informed consent on the second day after admission. Because of the EPEC detections took time, all of the diarrhoeal patients admitted had to be interviewed. All cases positive with EPEC should be included in the study as suspect cases with their answered questionnaires. The antibiotic sensitivity tests were carried out on EPEC strains to define patients as exposure and non-exposure cases.

3.3.3. Variable and definitions used in this study

- *Demographic information*

These are the factors concerned with demographics of suspect cases such as age, sex, living area, education, etc. These factors can also describe the distribution of diarrhoeal patients in Bac Ninh province during the time study.

- *Clinical data*

The clinical data of the patients can help to detect the outbreaks in the study area through the date of hospitalization of the suspect cases. However, some information in clinical data might be related with antibiotic resistance such as using drugs before admission, number of days with diarrhoea of patients, complications (if any), etc.

- *The medical history of suspect cases*

In this study, we included history of chronic illness, recent hospitalisation, recent out-patient clinic visits, recent history of diarrhoea, recent history of antibiotic treatment, and recent contact with diarrhoeal patient, etc.

A person, who had a chronic disease, was likely to have used one or more kinds of antibiotics in the past. It might have led to antibiotic resistance when the case later gets diarrhoea caused by bacteria

Similar to that, a history of antibiotics used for treatment might be related with antibiotic resistance.

Resent close contact with a diarrhoeal patient is one of the factors because the faecal - oral mode of transmission. Close contact with diarrhoeal patients (take care of one in hospital or at home) could be more likely than to contract disease from another patient.

- *Misuse of antibiotics among the public*

There are many factors concerning with misuse of antibiotics. Knowledge about antibiotics and their use is related with their habit in using antibiotics. In addition, their general knowledge about infectious diseases could be related too. So it could be an important variable.

According to the literature review, the habit of using drugs without a doctor's prescription was considered as an important risk factor associated with antibiotic resistance. Using drugs without doctor's prescription might lead to using antibiotics in a wrong way. On the other hand, using antibiotics for non-infectious diseases will lead to increasing antibiotic contamination of the environment.

- *Sanitation and hygiene conditions*

  Information on water supplies regarding drinking water was collected in this study as an important factor in transmission.

  The sanitation such as open sewage, open latrines are likely to contribute to the contamination of water sources. It might contribute to spread of antibiotic resistant strains from the patient to the environment.

- *Family economic level*

  The economic level of the household is related to the ability to buy drugs for treatment.

3.3.4. Microbiology method:

- *Stool culture:*

  Stool specimens were collected after diarrhoeal patients were admitted and before treatment started. 3 grams of stool was growing in Mac Conkey and SS agar (Difco - USA) at 35^{O}C - 37^{O}C for 18 - 24 hours [54]. In cases where the bacterium was not growing, the stool sample was enriched by passage in heart infusion broth at 35^{O}C - 37^{O}C for 16 - 18 hours and then sub-cultured on the agar plate. Smears from colonies in the agar plates were gram-stained and studied in the microscope. In cases where gram-negative bacilli were found, further prossessing was done with biochemical tests [54]. Confirmation of EPEC strains was done by agglutination tests with polyvalent antiserum for EPEC (Bio-Rad - USA).

- *Drug susceptibility testing by agar disk diffusion assay:*

  The disk diffusion method was performed on Mueller - Hinton agar with antibiotic disks according to the instructions of the manufacturers [45]. In this study, we chose 6 kinds of antibiotics, which are representative for the common

antibiotic groups in used in Bac Ninh province (following the statistical record of the pharmaceutics department of Bac Ninh hospital). These drugs can be bought very easily in the province. Some of them are not so expensive so the population can buy them for treatment with their financial resources.

Ampicillin belongs to a group of antibiotics called penicillins. They kill bacteria by interfering with their ability to form cell walls. The bacteria therefore break up and die. Ampicillin is a broad spectrum antibiotic which means it has the ability to kill a wide range of bacterial infections in the body [4].

Tetracycline contains the active ingredient tetracycline hydrochloride, which belongs to a group of antibiotics called the tetracyclines [4].

Ceftriaxone belongs to a group of antibiotics called the cephalosporins. Its mechanism of activity is the same as penicillin's group. Ceftriaxone is given by injection or infusion, and is generally used for severe infections [4].

Chloramphenicol is a powerful antibiotic with the ability to inhibit a wide variety of bacteria. It interferes with the production of proteins that the bacteria need to multiply and divide. This inhibits the ability of the bacteria to grow (bacteriostatic) and therefore stops the spread of the infection [4].

Trimethoprim/sulfamethoxazole is the two active compounds in co-trimoxazole. The drug belongs to the sulphonamide group of antibiotics. They work inside the bacterial cell, where they stop the manufacture of a necessary chemical for many bacteria called folic acid. Folic acid is necessary for the production of genetic material (DNA). Without the production of DNA the bacteria are not able to reproduce, and the spread of the infection is stopped (a bacteriostatic effect) [4].

Ciprofloxacin belongs to a group of broad spectrum antibiotics called the quinolones. It works by entering the bacterial cell and inhibiting a enzyme called DNA-gyrase which is involved in the organization of genetic material (DNA). This therefore prevents the bacteria from reproducing, and their growth is stopped. Ciprofloxacin is effective against several types of bacteria that tend to be resistant to other commonly used antibiotics. It is used to treat a wide range of infections, including infections of the chest, urinary tract and of the gastro-intestinal system. It is also used as a single-dose treatment for gonorrhoea [4].

In the antibiotic sensitivity test, the disks containing chloramphenicol (30µg), ampicillin (30µg), tetracycline (30µg), ceftriaxone (30µg), ciprofloxacin (30µg) trimethoprim-sulfamethoxazol (30/250µg) were placed on Mueller - Hinton agar plates, that were inoculated with bacteria to obtain semi-confluent grow. Inoculates were prepared by suspending colonies from blood agar plates incubated at 37^{O}C for 18 hours to achieve a density equivalent to McFarland 0.5 standard. The photometrically adjusted suspensions were swabbed on to the surface of the Mueller - Hinton agar plate. Each plate allowed by the application of 6 disks with antibiotic agents. The plates were incubated at 37^{O}C for 18 hours. Zones of inhibition were measured and recorded (by millimetre). The control strain used in all antibiotic sensitivity tests to check for accurate test performance was *Escherichia coli* ATCC 25922 [70,71].

3.4. Reliability and validity

3.4.1. Reliability

Reliability is an expression of the degree of agreement between repeated assessment of the same by the same or the other persons [72].

Misdiagnosis of EPEC was a source of concern. To solve this problem the EPEC strains were conformed at Enteric Pathogens Laboratory in NIHE (the reference laboratory in Vietnam).

In our study, we chose 6 medical doctors as interviewers from the selected departments. Relatively large number of interviewers might lead to some variation in the information collected. This was discussed after the pilot study, and the interviewers were required to ask questions in the same order and precisely report the answers.

Antibiotic sensitivity tests has been done in the Antibiotic Sensitivity Laboratory in NIHE (the reference laboratory in Vietnam).

3.4.2. Validity

Validity is an expression of the degree which a test is capable of measuring what it intends to measure [72]. For the detection of EPEC strain, we conducted a short training course about microbiology technique in the Microbiology Department of Bac Ninh provincial hospital to avoid misdiagnosis.

In the questionnaire, we designed clear and simple questions covering each point we were interested in elucidating. The questionnaires were translated to Vietnamese and interviews were conducted in Vietnamese to reduce the loss of information. We also carried out a pilot study before the main study was done. After the pilot study, we identified the questions which were not suitable or gave imprecise answers. This helped us to modify to a more suitable questionnaire.

3.5. Data handling and data analysis

After the data collection process was successfully completed, all the data were entered into the computer, and routine checking and data cleaning performed. The data was analysed with Epi-Info™ 6 software (Public Health Domain Software, CDC, Atlanta, Georgia, USA), and Software Package for Social, Sciences version 12.00 (SPSS for Window - Gorinchem, Netherlands).

Percentages and or proportions were calculated to show the distribution of the study population by socio-demographic characteristics. We will calculate the match of odds ratio (OR) of the difference between exposure and non-exposure groups with antibiotic resistance. All factors listed in the questionnaire were tested. The statistical significant level was set at P-value less than or equal to 0.05.

A conditional logistic regression analysis was performed to identify which of these potential risk factors ($P < 0.05$ in univaried analysis) remained independent variables associated with the antibiotic resistant situation [59,61].

3.6. Ethical consideration

Participants of this study were recruited totally on voluntary basis. They were neither forced nor persuaded to participate in the study, instead, once approached, it was upon themselves deciding whether or not to participate or provide information. Even those who initially accepted to participate were free to withdraw in the course of the study without any consequences. We explained the purpose of the study to each subject of the study and asked for permission to interview and collected specimens. The research team guaranteed the anonymity of the investigation and the confidentiality of the information provided.

Scientific merit: the result from the study will provide answers to the questions being addressed. Furthermore, result of this study could help health authorities to adapt policy to control this situation.

The study has been approved by the Regional Ethics Committee (REK-II) and the Ministry of Health (MOH), Hanoi, Vietnam. Submission of the project for ethical clearance has been done in Norway and to the Ministry of Health in Vietnam.

Feedback results: the results of the study will be reported to the doctors who participated in this study in Bac Ninh hospital, to the local community leaders, to the local and national health officials, to the funding agencies that supported the study, and to appropriate national and international scientific journals.

3.7. Time table

July 2004	
16 - 21	- Working with the leaders of NIHE, head of Bacteriology Department and make plant to carry out project - Select staff for working team in Enteropathogenic Laboratory of NIHE - Prepare media, chemical substances and equipments for working in laboratory
22 - 23	- Working with health authorities of provincial hospital in Bac Ninh Province - Design and pilot test record form and questionnaires. - Make arrangement for staff, training, equipment, transport, finance, and accommodation. - Training interview skill for interviewers.
26 - 28	- Training for staffs working in laboratory in B?c Ninh Hospital. - Draw up dally work plan for all staffs. - Pilot test for data collection and working in laboratory.
29 - 30	- Modification of questionnaires, as necessary. - Arrange for laboratory procedures. - Conduct staff seminar for reporting progress, discussion about problem, maintaining morale.
August 2004	- Data collection - Working in laboratory.

September 2004	-	Data collection
	-	Working in laboratory.
October 2004	-	Data collection
	-	Working in laboratory.
November 2004	-	Data collection
	-	Working in laboratory.
December 2004	-	Data collection
	-	Working in laboratory.
Jan. 05 - Feb. 05	-	Entry data into the computer.
	-	Data compilation and analysis
March - May	-	Writing thesis.
June 2005	-	Thesis defence

CHAPTER IV: STUDY RESULTS

4.1. Characteristic of the study sample

There were 526 diarrhoeal patients, according to our case definition, admitted to the Bac Ninh hospital during the period of this study. All of the diarrhoeal patients were examined and treated in Paediatric Department, General Enterology Department, or Communicable Diseases Department. Stool samples were collected to identify the causative agent of diarrhoea. EPEC infections were confirmed from 227 cases. They were included: 56 cases from General Enterology Department, 153 cases from Paediatric Department, and 18 cases from Communicable Diseases Department.

4.1.1. Geographic distribution:

The first case was reported on 1st August 2004 (week 33). At that day we found 3 cases positive for EPEC, all of them were children under 5 years of age. The last case was reported on 24th December 2004 (week 52) (***figure 6***).

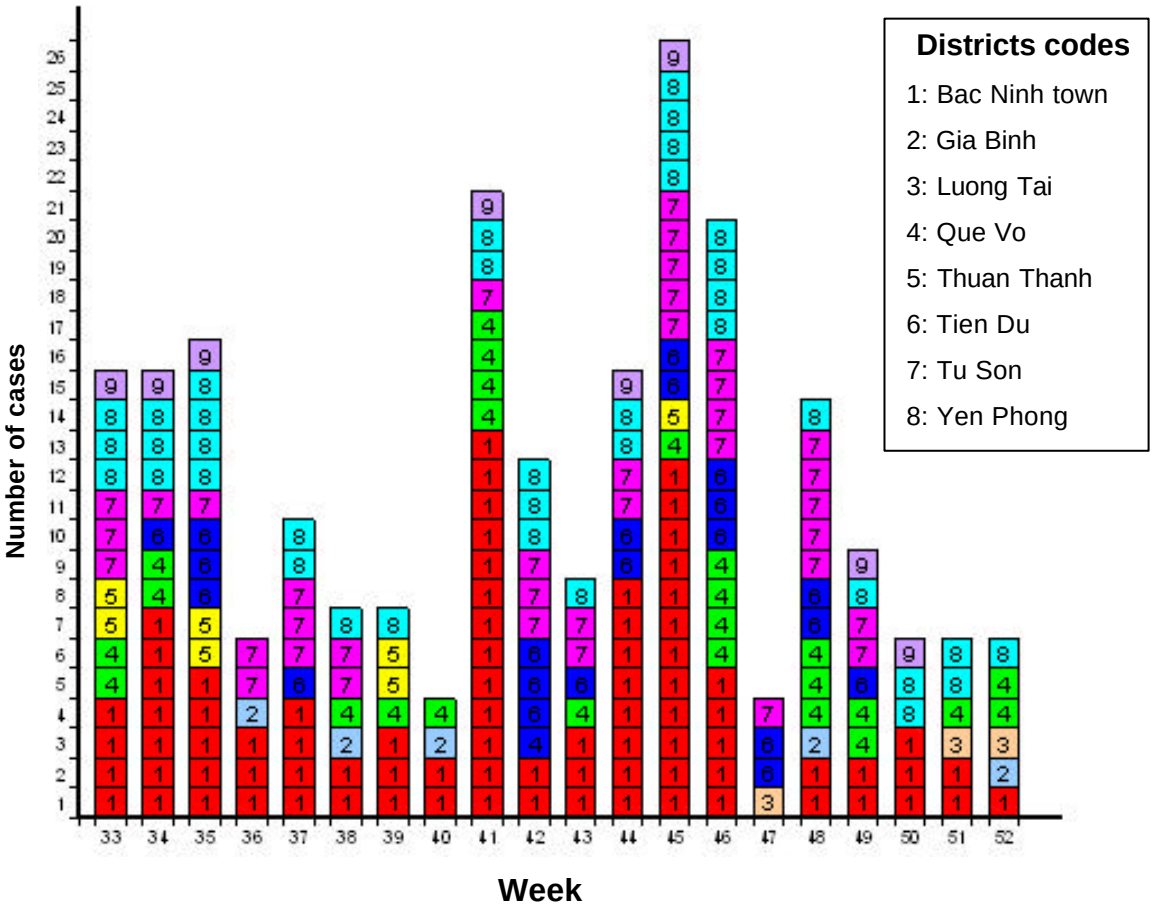

***Figure 6**: Distribution by week and district*

Among the total 227 cases, there were 219 cases living in 8 districts of Bac Ninh province: 37.90% (83/219) in Bac Ninh town; 2.28% (2/219) in Gia Binh; 1.37% (3/219) in Luong Tai; 11.87% (26/219) in Que Vo; 3.20% (7/219) in Thuan Thanh; 9.59% (21/219) in Tien Du; 16.89% (37/219) in Tu Son; and 16.89% (37/219) in Yen Phong (***table 6***). The other cases were coming from other places.

The diarrhoeal patients caused by EPEC occurred every week during the period of the study. The highest number of cases in a week occurred in the 45th week (26 cases). It seemed there were some outbreaks in Bac Ninh town from 33rd week to 35th week; in 41st week; and from 44th to 46th weeks

Table 6*: Distribution of cases by districts.*

	District	***Number of cases***	***Population***	***Incident rate (%)***
1	Bac Ninh town	83	80726	0.103
2	Gia Binh	5	102395	0.005
3	Luong Tai	3	102797	0.003
4	Que Vo	26	154915	0.017
5	Thuan Thanh	7	143192	0.005
6	Tien Du	21	130661	0.016
7	Tu Son	37	122673	0.030
8	Yen Phong	37	145534	0.025
	Total	219	982893	0.022

Actually, follow the data collected from Bac Ninh hospital during the period of our study showed that the attack rate in Bac Ninh town (0.103%) was higher than another places in Bac Ninh province.

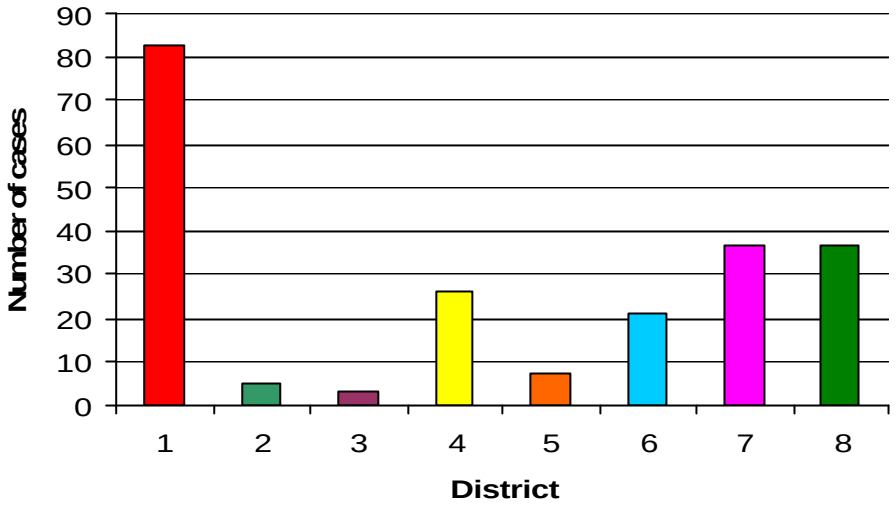

Figure 7: *Distribution of cases by district*

4.1.2. Sex distribution:

The sex ratio (male/female) was 1.64, higher among males than females. In total, there were 136 (62.10%) male and 82 (37.90%) female cases in our study. Except in three districts with too few cases (Gia Binh, Luong Tai and Thuan Thanh), there was not a significant difference in sex distribution between males and females in Bac Ninh town; but other districts had a higher percentage of male patients than Bac Ninh town (***table 7***).

Table 7: *Sex distribution by districts*

	District	Number of cases	*Sex*	
			No. Male (%)	*No. Female (%)*
1.	Bac Ninh Town	83	***43*** (51.81%)	***40*** (48.19%)
2.	Gia Binh	5	***3*** (60.00%)	***2*** (40.40%)
3.	Luong Tai	3	***2*** (66.67%)	***1*** (33.33%)
4.	Que Vo	26	***18*** (69.23%)	***8*** (30.77%)
5.	Thuan Thanh	7	***5*** (71.43%)	***2*** (28.57%)
6.	Tien Du	21	***18*** (85.71%)	***3*** (14.29%)
7.	Tu Son	37	***24*** (64.86%)	***13*** (35.14%)
8.	Yen Phong	37	***23*** (62,16%)	***14*** (37.84%)
	Total	**219**	**136** (62.10%)	**83** (37.90%)

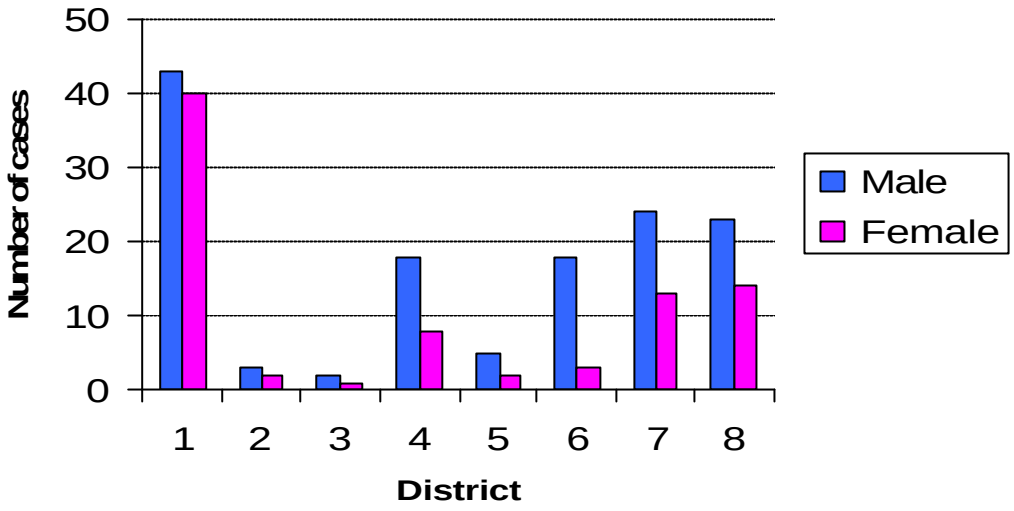

Figure 8: *Sex distribution by districts*

4.1.3. Age distribution: *(for both exposure and non-exposure)*

Table 8*:* *Age distribution*

	Ages	No. of cases	Sex	
			Male	Female
1	**Under 5 years**	149(68.04%)	101	48
2	Group 5 to 9 years	5 (2.28%)	5	1
3	Group 10 to 14 years	5 (2.28%)	3	2
4	Group 15 to 19 years	8 (3.65%)	5	3
5	Group 20 to 24 years	6 (2.74%)	3	3
6	Group 25 to 29 years	3 (1.37%)	2	1
7	Group 30 to 34 years	7 (3.20%)	3	4
8	Group 35 to 39 years	2 (0.91%)	1	1
9	Group 40 to 44 years	5 (2.28%)	2	3
10	Group 45 to 49 years	3 (1.37%)	0	3
11	Group 50 to 54 years	7 (3.20%)	2	5
12	Group 55 to 59 years	6 (2.74%)	2	4
13	Group 60 to 64 years	2 (0.91%)	1	1
14	Group 65 to 69 years	3 (1.37%)	1	2
15	Group 70 to 85 years	8 (3.65%)	4	4
	Total	**219 (100%)**		

Most of the cases (149/219 - 68.04%) were among children under 5 years of age. The age distribution of cases is described in the ***table 8***. In this study we could see the cases of EPEC infection in all age groups.

There was difference in sex distribution in the age group under 5 years of age (101 male / 48 female) and the group 5-9 years (5 male / 1 female). In the other age groups no deference was found.

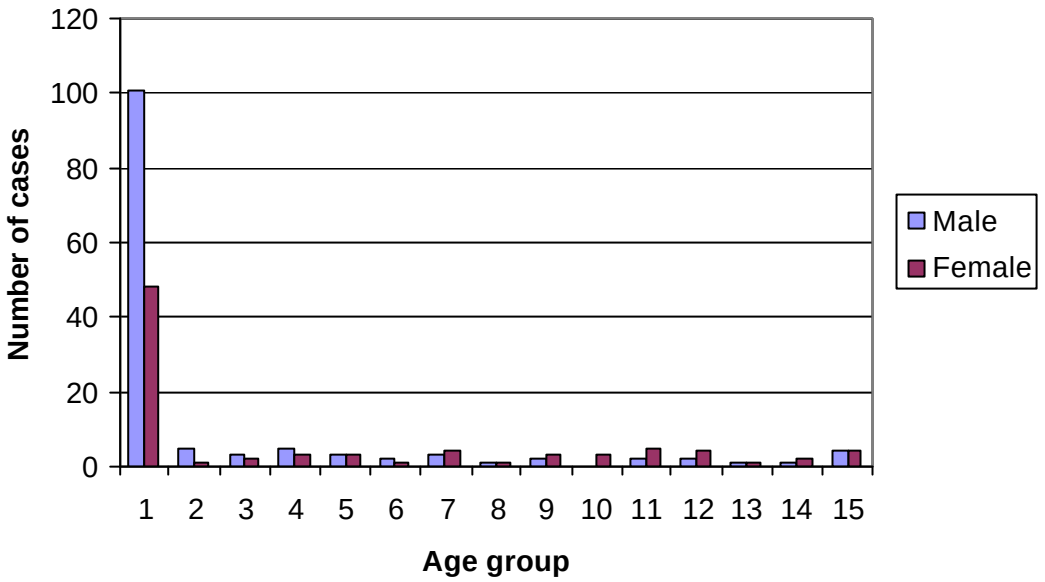

***Figure* 9**: *Distribution of sex by age groups*

4.2. Clinical manifestations:

In total, there were 44 (20%) cases with diarrhoea lasting for more than 7 days. There were 112 (51.14%) cases with vomit and 46 (21%) cases with fever. Only 18 (8.22%) cases reported headache.

Treatment before admission: 101 (46.12%) cases had self treatment with antibiotics before they were admitted to the hospital. During the time they stayed in the hospital, 198 (90.41%) cases were treated with antibiotics.

There were 5 cases which had complications after treatment in hospital with symptom such as blood in their stools. All of them were children under 5 years of age.

Some risk factors associated with the patients' history: 87 cases had recently been hospitalized; 121 cases had diarrhoea during the last 90 days; 131 cases had a history of antibiotic treatment during the last 90 days before admission.

4.3. Univariate analysis:

The result of the univariate analysis of risk factors associated with antibiotic resistance in this study is summarised in ***table 9***

In our study, 122 (71.35%) of exposure cases compared to 27 (56.25%) of non-exposure cases were children under 5 years of age (OR=1.94; 95%CI: 0.95-3.94; P<0.05). The difference between this age group with the other age groups is significant.

4.3.1. The relationship with clinical data

Number of days the patients had diarrhoea was significant. 27 (15.79%) of exposure cases had had diarrhoea longer than or equal 7 days, while 17 (35.42%) of non-exposure cases with the same situation (OR=0.34; 95%CI: 0.16-0.75; P<0.01). It means that longer lasting diarrhoea was a risk factor in association to the antibiotic resistance.

History of use of antibiotics before admission to the hospital (OR=2.52; 95%CI: 1.20-5.33; P<0.01), having chronic underlying disease (OR=0.40; 95%CI: 0.17-0.90; P<0.02), having used antibiotics during the last 90 days (OR=2.59; 95%CI: 1.28-5.25; P<0.01), use of antibiotics by a family member during the last 90 days (OR=2.02; 95%CI: 1.01-4.07; P<0,03) and history of visit to another patient in a hospital or a clinic recently (OR=8.43; 95%CI: 1.17-171; P<0.02) were associated with the risk of carrying antibiotic resistant EPEC.

4.3.2. The relationship with antibiotic consumption

In total, 92 (53.80%) of exposure cases and 11 (22.92%) of non-exposure cases usually went directly to private pharmacy (OR=2.79; 95%CI: 1.13-6.95; P<0.02), some of the cases had decided to treat themselves (OR= 0.19; 95%CI: 0.04-0.88; P<0.02). The knowledge of the patients (or the people, who were take care of the patient under 5 years of age) was also related to antibiotic resistance in this study (OR=3.77; 95%CI: 1.79-8.02; P<0.01).

140 (81.78%) of exposure cases compared with 18 (37.50%) of non-exposure cases (OR=7.53; 95%CI: 3.53-16.18; P<0.01) usually took an antibiotic when they were getting sick for any reason.

When asked, 143 (83.63%) of exposure cases and 33 (68.75%) of non-exposure cases (OR=2.32; 95%CI: 1.05-5.13; P<0.03) answered that they often bought the drug for treatment in private pharmacies; some of the cases bought the drug from street vendors (OR=0.17; 95%CI: 0.05-0.62; P<0.01). 111 (64.91%) of exposure cases and 22 (45.83%) of non-exposure cases often bought the antibiotic without doctor's prescription (OR=2.19; 95%CI: 1.09-4.40; P<0.02).

In case they did not have a doctor's prescription, they usually bought the antibiotic following the advise of the drug seller (OR=1.98; 95%CI: 0.99-3.97; P<0.04). The other behaviour associated with antibiotic resistance was using antibiotic for non-infection diseases. When they were getting flu (OR=2.28; 95%CI: 1.11-4.71; P<0.02) or getting diarrhoea (OR=2.99; 95%CI: 1.41-6.44; P<0.01), even if they did not know the reason, they would also take antibiotics.

In some cases, antibiotics are not so cheap. In the opinion of the interviewees, they thought that the price of antibiotics was extremely high (OR=0.42; 95%CI: 0.17-1.04; P<0.04). Their methods to solve this problem was to reduce the dose (OR=0.44; 95%CI: 0.21-0.91; P<0.02); or/and stop using the drug immediately after the symptoms were reduced (OR=1.96; 95%CI: 0.98-3.94; P<0.04). Using too low of antibiotics became a big problem. This is one of the phenomena which is associated to antibiotic resistance of bacteria.

4.3.3. The factors relationship with the dwelling conditions

The habit of drinking un-boiled water among the rural people may have been increasing the number of people getting diarrhoea. It had a significant impact on antibiotic resistance (OR=6.58; 95%CI: 1.00-277.8; P<0.03).

Using an unhygienic toilet (OR=2.15; 95%CI: 1.06-4.40; P<0.03) and disposal of sewage directly to the environment (OR = 2.03; 95% CI: 1.00-4.15; P<0.04) were associated with antibiotic resistance in the study population.

4.4. Multivariate analysis

The result of the multivariate analysis is shown in the ***table 10***. In the multivariate analysis, the choice of risk factors to put in to the model was made by a "step by step" process. We chose the risk factors that were significantly associated with presence antibiotic resistance of the EPEC isolates in the univariate analysis.

- Age group under 5 years of age.
- Diarrhoea lasting longer than or equal to 7 days.
- A history of having used antibiotics before admission to the hospital.
- Using antibiotics regularly when getting flu and diarrhoea.
- Having a chronic underlying disease.
- A history of antibiotic treatment in last 90 days.
- A history of using antibiotics by a family member in last 90 days.
- A history of a recent visit to another patient in a hospital or clinic
- Going directly to private pharmacy when getting sick.
- Taking an antibiotic when they were getting sick from any reason.
- Buying the antibiotic without a doctor's prescription.
- Buying the drug in private pharmacies.
- Following the advise of the a drug seller.
- Stopping using the drug immediately after reduction of symptoms.
- Reducing dose when they did not have enough money to buy the drug.
- General knowledge of infectious disease of the patients or the guardian of the patient under 5 years of age.
- Drinking un-boiled water.
- Using unhygienic toilet.
- Disposal of sewage directly into the environmental.

Some variables were not considered for this model such as if the patients had decided to treat themselves; if they had bought the drug in the street vendors; and

the price of antibiotics. Even though these were significant in the unvariate analysis, these phenomena concerned only few cases.

Table 10: *Result of the multivariate analysis*

Potential risk factors	Matched OR	95% CI	P-value
Age group under 5	1.43	0.30 - 6.86	0.652
Diarrhoea greater or equal 7 days	**0.21**	**0.05 - 0.93**	**0.039**
Use antibiotic before hospitalisation	2.21	0.66 - 7.39	0.197
Have chronic disease	0.74	0.17 - 3.24	0.690
History use antibiotic in last 90 day	**5.41**	**1.39 - 21.00**	**0.015**
History use antibiotic of family member	0.44	0.11 - 1.83	0.259
History visit patient in hospital	2.60	0.15 - 45.64	0.513
Self treat when get sick	0.06	0.003 - 1.16	0.063
Go to pharmacy when get sick	3.79	0.96 - 14.87	0.056
General knowledge of people	**0.15**	**0.04 - 0.52**	**0.003**
Using antibiotics often when get sick	**5.74**	**1.70 - 19.34**	**0.005**
Often buy drug in street vendor	0.34	0.02 - 5.54	0.448
Often buy drug in private pharmacy	2.85	0.67 - 12.23	0.158
Buy antibiotic without prescription	0.63	0.05 - 8.94	0.734
Buy antibiotic follow advice of seller	1.41	0.12 - 17.08	0.785
Using antibiotics often when get flu	**9.92**	**2.42 - 40.73**	**0.001**
Using antibiotics often when get diarrhoea	**6.27**	**1.52 - 25.85**	**0.011**
Stop use antibiotic when reduce symptom	1.97	0.43 - 9.11	0.387
Stop use antibiotic after 1 week	0.65	0.09 - 4.59	0.667
Price of antibiotic is expensive	0.52	0.09 - 3.02	0.469
Price of antibiotic is acceptable	1.10	0.14 - 8.50	0.925
Reduce dose if not enough money	**0.17**	**0.05 - 0.59**	**0.005**
Drinking un-boiled water	**40.38**	**1.44 - 1131.46**	**0.030**
Use unhygienic toilet	0.84	0.24 - 2.954	0.780
Disposal sewages unhygienic	0.83	0.25 - 2.797	0.768

In the multivariate analysis, when we used conditional logistic regression; many factors remained independently associated with antibiotic resistance. Eight of these, such as having diarrhoea lasting longer than or equal to 7 days; a history of

use of antibiotics during the last 90 day; general knowledge of infectious diseases; using antibiotic when getting sick for any reason; using antibiotic when getting diarrhoea; using antibiotic when getting flu; drinking un-boiled water; reducing dose if not enough money; are factors contributed to increasing the risk of antibiotic resistance.

The other factors that were risk factors according to the univariate analysis (***table 9***). However, these factors did not remain independently associated with antibiotic resistance.

4.5. Laboratory results

From the 1st of August to the 31st of December, 2004, a total of 526 diarrhoeal patients admitted to the Bac Ninh hospital were suspected cases according to our cases definition. Of which, 227 cases were positive with EPEC. These were confirmed by culture, biochemical tests and agglutination test with polyvalent antiserum for EPEC. There were 219 cases living in Bac Ninh province.

All of them were isolated from stool specimens. In addition, we did not find any other kind of the enteric pathogenic bacteria such as *V.cholerae; Salmonella,* or *Shigella* from the patients admitted.

4.6. Resistant patterns of EPEC strains isolated

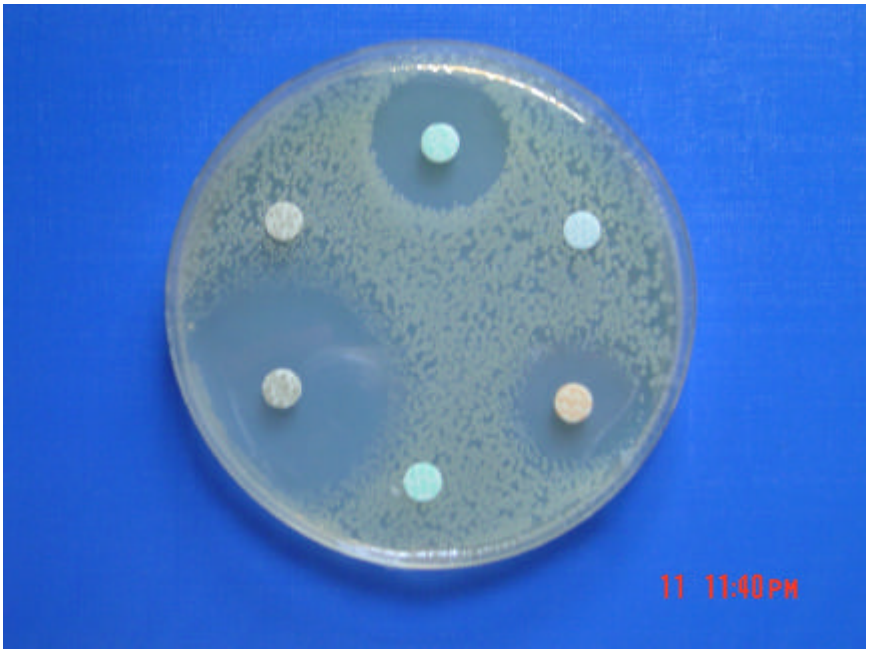

Figure 10: *Antibiotic sensitivity test by disk diffusion method*

The six antibiotic agents were used in this study, included ampicillin, tetracyline, chloramphenicol, trimethoprim/sulfamethoxazol, ceftriaxone and ciprofloxacin. The following control strain was used in all tests to check for accurate test performance: *E.coli* ATCC 25922. The disk diffusion method was used to evaluate antibiotic sensitivity of the EPEC strains.

In this study, there were 48/227 strains (21.15%) sensitive with all of 6 antibiotics (non-exposure cases) and the other strains (78.85%) were resistant to at least one kind of the antibiotics tested. Among the antibiotic resistant strains, 22 strains (12.29%) were resistant to one antibiotic agent, 63 strains (35.20%) were resistant to two antibiotics, 45 strains (25.14%) were resistant to three antibiotics, 42 strains (23.46%) were resistant to four antibiotic and 7 strains (3.91%) were resistant to five antibiotics. There was no strain resistant to all antibiotics tested.

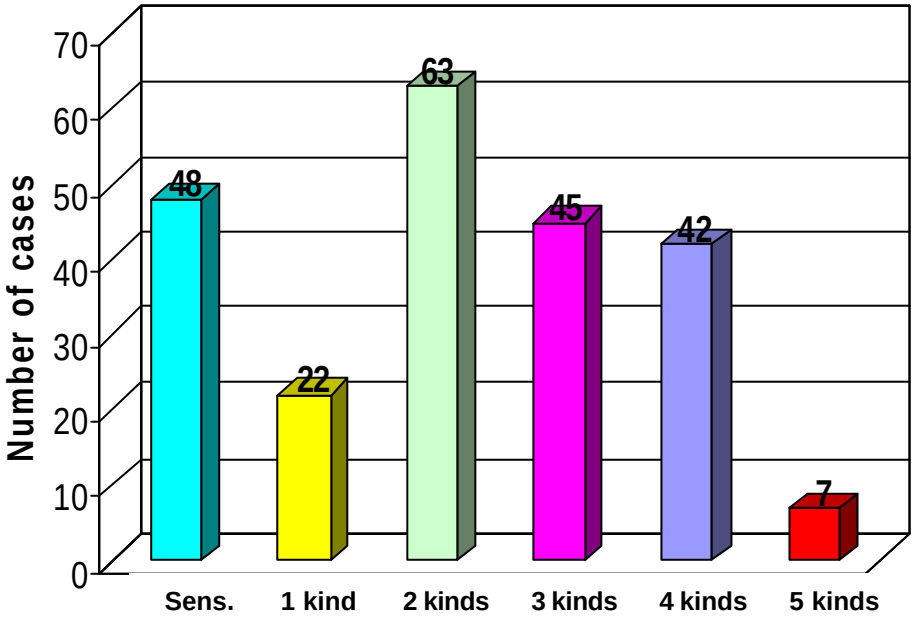

***Figure 11**: Distribution of cases by number of antibiotic resistant present*

- *Ampicillin:*

157 strains (69.16%) were resistant to ampicillin; 6 strains (2.65%) were sensitive at intermediately sensitive; and 64 (28.19%) were fully sensitive.

- *Tetracylin:*

  101 strains (44.49%) were resistant to tetracylin; 4 strains (1.76%) were sensitive at intermediately sensitive; and 122 strains (53.75%) were fully sensitive.

- *Chloramphenicol:*

  65 strains (28.63%) were resistant to chloramphenicol; 9 strains (3.96%) were sensitive at intermediately sensitive; and 153 strains (67.41%) were fully sensitive.

- *Trimethoprim/Sulfamethoxazol:*

  154 strains (67.84%) were resistant to trimethoprim/sulfamethoxazol; 3 strains (1.32%) were sensitive at intermediately sensitive; and 70 strains (30.84%) were fully sensitive.

- *Ceftriaxone:*

  7 strains (3.08%) were resistant to ceftriaxone; 10 strains (4.41%) were sensitive at intermediately sensitive; and 201 strains (92.51%) were fully sensitive.

- *Ciprofloxacin:*

  9 strains (3.96%) were resistant to ciprofloxacin; 2 strains (0.88%) were sensitive at intermediately sensitive; and 216 strains (95.15%) were fully sensitive.

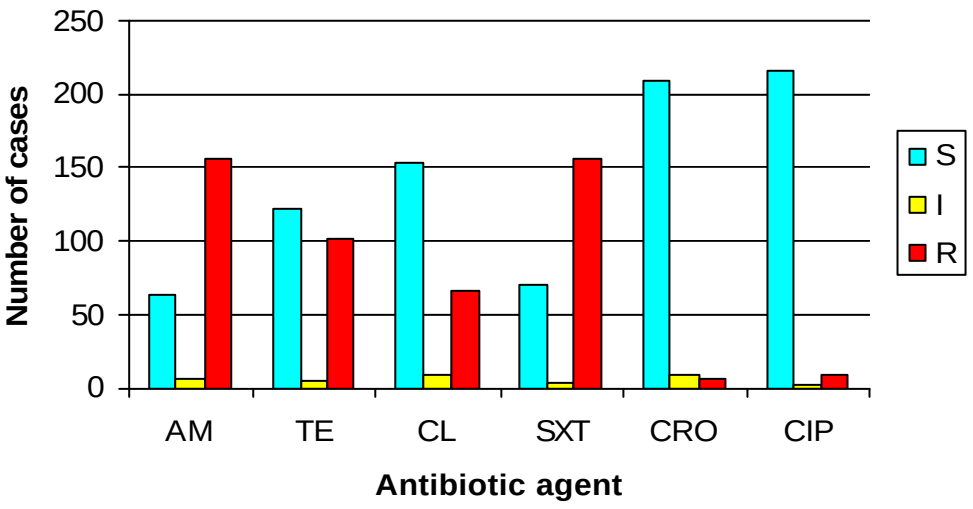

***Figure 12**: Distribution of resistance by antibiotic*

***Table 11**: Result of antibiotic sensitivity testing (disk diffusion method)*

Antibiotic	***Resistant***		***Intermediately sens.***		***Sensitive***	
	n	(%)	n	(%)	n	(%)
AM	157	69.16%	6	2.65%	64	28.19%
TE	101	44.49%	4	1.76%	122	53.75%
CL	65	28.63%	9	3.96%	153	67.41%
SXT	154	67.84%	3	1.32%	70	30.84%
CRO	7	3.96%	10	4.41%	210	92.51%
CIP	9	3.96%	2	0.88%	216	95.15%

CHAPTER V: DISCUSSION

In different geographic areas, the risk factors associated with carrying an antibiotic resistant EPEC in a diarrhoeal case may be different. It may be related to many factors such as spread of resistant strains; the use of antibiotics in the community, environmental hygienic conditions, etc. The question is: “What are the local risk factors related to the antibiotic resistant situation in the study place?”

In this study, we found that some risk factors were statistically associated with carrying an antibiotic resistant EPEC among diarrhoeal patients in Bac Ninh hospital. These factors included age group and the duration of diarrhoea; the behaviour related to buying and using antibiotics; and environmental factors.

5.1. Strength of the study

In this study, we used a hospital - based detection to select the diarrhoeal cases and collect stool samples. After that, we used standard methods from National Institute of Hygiene and Epidemiology (NIHE) which have been used in Viet Nam to detect EPEC from stool specimens. Bac Ninh hospital is a provincial hospital. The staffs of the hospital have good working skills and experience in both clinical diagnostic and laboratory work. After specific training for the purpose of this study they could work independently and followed the standard procedure to detect EPEC from stool specimens. Positive isolates were reconfirmed by the Enteropathogens Laboratory of NIHE. The study place was not so far from Ha Noi, so it was easy to carry out the study and co-ordinate the reference laboratory of NIHE and Bac Ninh hospital.

The epidemiologic information was collected through interviews with questionnaires. Interviewers used Vietnamese language; all of the cases were ethnically King who use the Vietnamese language; and the questionnaires had been translated by a special experienced translator who has a good command of in English and also of community health. That ensured that the target study was understood and questions answered according to a correct understanding of the questionnaire. Training for interviewers and a pilot study had been performed before the data collection was carried out. After the pilot study, the questionnaire was revised and made more appropriate for the local situation. The reports from the study place to NIHE and feedback information were exchanged regularly. The

interviewers re-checked the questionnaires after interviewing at a suitable time of the same day to identify missing answers.

5.2. Weakness of the study

In our study, the time was limited. The field trip was conducted during five months. Thus it was difficult to reach the pre-set sample size of 236. In Northern Vietnam, the rainy season (wet season) starts in May and stops at the end of August. The wet season is also the main season for enteric diseases cause by enterobacteria. The study was carried out in the last month of the wet season. That was why we could not collect enough cases.

Bac Ninh hospital is a provincial hospital, located in Bac Ninh town. The hospital not only receives the patients from all districts of Bac Ninh province but also receives patients from outside. On the other hand, it is a difficult transport situation in rural areas, so some of the diarrhoeal patients could not come to Bac Ninh hospital or just went to the district hospitals when they were getting acute diarrhoea in local areas. It is likely that a number of cases in the community were not recorded from Bac Ninh hospital. It may explain why the number of cases from Bac Ninh town was much higher than from the other districts. Therefore, our study cannot accurately state the incidence of antibiotic resistance in the district population.

Some risk factors might have been difficult to identify. For example, it was very difficult to define the economic level of the study subjects. Some time they could not calculate their income exactly; and household goods and number of domestic animal could only a serve as proxies for the economic level.

Finally, we just could identify EPEC isolates by polyvalent antiserum because of a limited budget. We did not have enough monovalent antiserum for identification of the collected strains more exactly.

5.3. Characteristic of the sample

There are very few epidemiological studies on diarrhoeal disease cause by EPEC. In this study, all included patients from the districts reported positive with EPEC.

5.3.1. Temporal distribution

E.coli is the predominant facultative anaerobe of the human colonic flora. Diarrheagenic *E.coli* strains have acquired different sets of virulence genes and thus differ from those normally resident in the colon in possessing distinct virulence factors. They are distinguished and defined on the basis of their pathogenic mechanisms and have been divided into six well-defined pathogenic groups: enteroinvasive (EIEC), enterotoxigenic (ETEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroaggregative (EAggEC), and diffusely adhering (DAEC) *E.coli* [73].

E.coli is a normal member of the faecal flora. It can survive and replicate in many different non-host environments such as water, soil, sediments, sand, etc [74,75,76,77]. *E.coli* has been known to thrive not only in warmer, sub-tropical waters, but also in temperate waters. A report showed that *E.coli* was the cause of food poisoning (potato salad) and brought about a big diarrhoeal outbreak, which affected to over 4,500 people in Chicago, Illinois in June 1998 [78].

EPEC is a pathogenic *E.coli* that is responsible for diarrhoeal disease. It is important among diarrhoeagenic *E.coli* that has been linked to infant diarrhoea in the developing countries [54,55].

Diarrhoeal patients due to EPEC came to the hospital every week during the period of the study. We observed some increasing of diarrhoeal cases in Bac Ninh town from 33rd week to 35th week (the first half of August); in 41st week (the end of September); and from 44th to 46th weeks (the middle of October) (***figure 6***). In the study period, it was still rainy with high temperatures (29^{O}C - 31^{O}C) until the end of October. In addition, October was the beginning of wedding season, and following traditional custom of Vietnamese, wedding parties should be organised. In general, the hygienic condition in the local area, especially in rural areas, was not good. The wedding parties were the convenient condition for food poisoning. These might be the cause of the observed increase in diarrhoeal cases.

5.3.2. Age and sex distribution

In our study, 62.1% of cases were males and 37.9% were females. Most of the cases were among children under 5 years of age (68.04%). It was similar to

some another studies [54,55,79]. There was not so large a difference between male and female in Bac Ninh town, but more so in the rural areas such as Que Vo, Tien Du, Tu Son, and Yen Phong (***figure 8***). In addition, a disparity between male and female in the age group under 5 was clearly seen (101/48) (***figure 9***). In Bac Ninh town, a chief town of the Bac Ninh province, inhabitants have many opportunities to access information about hygiene and child care via the media such as books, television, radio, newspapers, etc.

In rural areas, especially in four districts with high frequency of diarrhoeal cases (Que Vo, Tien Du, Tu Son and Yen Phong), 83 cases (68.6%) were males and 38 cases (31.4%) were females. It might be two reasons for this situation. Toward the child cases, parents did not have much time to take care of children. The older children often take care of younger children within their family. A male child is usually more active than a female child; and could reach and eat many things. So the male child could get more diarrhoea than the female child. Toward the adult cases, because of behaviour such as eating outside, drinking water from unreliable source while going out to work might be more likely to occur in males. That could explain the different rate between male and female cases in rural areas.

5.4. Local risk factors

5.4.1. Diarrhoea long lasting

In our study, 44 (20.09%) of cases had had diarrhoea for 7 days or more. One case was recorded with 13 days with diarrhoea before admission to the hospital. In multivariate analysis, there was a significant difference between two groups exposure and non-exposure in the cases with diarrhoea equal or greater than 7 day (OR=0.21; 95%CI: 0.05-0.93; P<0.04). This might be related to uncontrolled antibiotic use. In Viet Nam, using antibiotics when getting sick is very common, especially in rural areas. They may use antibiotic to treat any kind of diseases. In addition many people did not know how to use them appropriately. Normally, when getting sick, people used drugs without doctor's prescription [15,16,18]. So this might contribute to antibiotic resistance.

5.4.2. General knowledge of people in infections

In our study, lack of general knowledge among people in infections was a risk factor for antibiotic resistance. 118 (53.88%) of all cases did not know the signs of infectious disease. Following the multivariate analysis, there is a significant difference between the two groups - knowing and not knowing the signs of infectious disease - (OR=0.15; 95%CI: 0.04-0.52; P<0.01). This factor has not been reported before this study was carried out. Lack of knowledge is usually related to the lack of information and poverty. This problem is more serious in rural areas. Lack of knowledge about the signs of infectious disease, which was concerned with misuse of antibiotic for treatment; uncontrolled use of antibiotics; and unhygienic condition have led to increasing antibiotic resistance.

5.4.3. The risk factors related to using antibiotic in the past

Using antibiotics in the communities was very common and uncontrolled. The people usually used it for many reasons when they were getting sick. Lack of knowledge of the signs of infectious diseases led to incorrect use of antibiotics.

People usually stopped using antibiotics when symptoms were reduced. When people did not have enough money to buy the full course, they just bought a part and reduced the member or size of the doses.

5.4.4. The risk factors related to misuse of antibiotic

158 cases within this study usually took at least one kind of antibiotic when they were getting sick for any reason. Of which, 106 cases took antibiotics when getting flu and 103 cases took antibiotics when getting diarrhoea. Flu is a disease caused by virus and it does not need treatment with antibiotics (except co-infection cases with bacteria); and diarrhoea, especially in children, might be caused by rotavirus. In that case, it does not need treatment by antibiotics. Using antibiotics on wrong medical indications could affect to bacteria in the intestine of flora, and make them become antibiotic resistant. Resistance might be spread to the environment; other bacteria could acquire them and become resistant.

5.4.5. Drinking un-boiled water

In many areas of the world, many parts of the population do not have access to clean potable water. In others, where public water supplies are available, system failures can place entire communities at risk [80]. EPEC is an important cause of diarrhoea especially in children in developing countries; it is acquired principally by ingestion of contaminated food or water [80].

In this study, although the numbers of cases associated with drinking un-boiled were not so many, but according to the univariate and multivariate, it was a risk factor associated with antibiotic resistant situation in Bac Ninh.

5.5. Resistance patterns of EPEC strains

Resistance to antibiotics is highly prevalent in bacterial isolates worldwide, particularly in developing countries [21,23,81,82]. Normal intestinal flora is a reservoir for resistance genes; the prevalence of resistance in commensal *E.coli* strans is a useful indicator of antibiotic resistance in bacteria in the community [83,84]. In addition, *E.coli* strains efficiently exchange genetic material with pathogens such as *Salmonella, Shigella, Yersinia*, etc, as well as with pathogenic *E.coli* [85]. In recent years, bacterial resistance to the ß-lactam antibiotic group has risen dramatically among human bacterial pathogens.

Because of limited budget, we just used 6 kinds of antibiotics to test the resistance patterns of EPEC strains isolated from diarrhoeal patients in this study. There were 179 cases (78.85%) resistant to at least one kind of antibiotic. Of which, 157 cases (69.16%) were resistant to two kinds or more of antibiotics. There was a high rate of resistant to ampicillin (69.16%) and trimethoprim-sulphamethoxazole (67.84%). This result is similar to some other studies [86,87,88].

9 isolates were also resistant to ciprofloxacin, a broad-spectrum antibiotic belonging to the antibiotic group called quinolones, which might reflect the increased use of these antibiotics in Viet Nam. This problem had been reported in another study with ETEC strains [89]. Use of fluroquinolones in food animals is also prevalence in many Asian countries. As a result of the wide use of antibiotics, an emerging prevalence of resistance to the newer antibiotics was found among faecal *E. coli* from adult individuals in Asian and South America countries [90]. For the emergence of *E. coli* quinolone resistance in Viet Nam there might be some

explanations: in Viet Nam, ciprofloxacin were used for the treatment of typhoid fever, and some infectious diseases such as respiratory infections, especially in hospital. Ciprofloxacin is also often used to prevent infections before and after operations. One survey conducted in Viet Nam indicated that less than 1% of customers came to drug stores with prescription and 94.9% decided by themselves which drugs to buy and some of antibiotic were given for 2.5 days or less [91].

CHAPTER VI: CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

- Antibiotic resistance has become an important problem in Bac Ninh province. There is a high rate in EPEC strains isolated from diarrhoeal patients that proved resistant to antibiotics. Mono-resistance to an antibiotic was a rare phenomenon in this.
- Multidrug resistance (resistance with greater than or equal to two kinds of antibiotics) exists a serious problem in Bac Ninh province.
- There are some cases of resistance to ciprofloxacin, a relatively new broad spectrum antibiotic, observed in this study
- Using antibiotics without a doctor's prescription, using drugs on wrong medical indications are very common in Bac Ninh province, especially in rural areas.
- Lack of knowledge about the signs of infectious disease, having a recent history of used antibiotics was related to presence of antibiotic resistant EPEC in Bac Ninh province.
- The habit of drinking un-boiled water and unhygienic condition in living area were associated with presence of antibiotic resistant EPEC in Bac Ninh province.

6.2. Recommendations

- Provide information about the danger of antibiotic resistance; manifestations of infectious diseases, evil influences of using antibiotic incorrect through multi-media such pamphlets, loudspeakers, local papers, local TV programmes, etc.
- Guide and encourage patients to use drugs according to the doctor's prescription, especially used antibiotic for treating infectious diseases.
- Pharmacies should sell drugs only following the doctor's prescription. It must be a rule for pharmacies, especially in private pharmacies.

- Provide safe water supplies and safe sewage disposal should be attached. In addition, the hospital infection control must be improved; medical sewage must be treated.
- Encourage the population to use boiled water. The hygienic condition in the communities should be improved, particularly in hot and rainy season.
- It is very important to monitor and control resistance to antibiotic of bacteria, especially entero pathogenic bacteria in Bac Ninh (in particular) and in rural areas (in general) of Vietnam.
- Antibiotic sensitivity test should be carried out in the health care centres (at least in the hospitals) to ensure using suitable antibiotic for treating infectious diseases.
- To enquire further, the resistant pattern of entero pathogenic bacteria should be detected with much more antibiotics.

REFERENCES

1. Absolute Astronomy Reference. Antibiotic. Available from: URL: www.absoluteastronomy.com.encyclopedia/a/an/antibiotic.htm
2. Neu HC. The crisis in antibiotic resistance. Science 1992;257:1064-73.
3. Shear P. A review of bacterial resistance to antimicobial agents in tropical countries. Ann Trop Paediatr 1993;13:219-26.
4. Bessard MM, Bechtel V, Moulin ML, Escousse MM, Nicot M, éditeur. Cours National de Phamacologie. Marketing ed. Paris: L'A.C.T.E.P; 1983.
5. Antibiotic and antibiotic resistance. Available from URL: http: // www.worldhistory.com
6. Calva JJ, Sifuentes-Osornio J, Ceron C. Antimicrobial resistance in fecal flora; longitudinal community-based surveillance of children from urban Mexico. Antimicrob Agent Chemother 1996;40:1699-702.
7. Sandvang D, Aarestrup FM, Jensen LB. Characterization of intergrons and antibiotic resistance genes in danish multiresistant *Salmonella enterica typhimurium* DT104. FEMS Microbiol Lett 1997;160:37-41.
8. Alangaden GJ, Lerner SA. Overview of antimicrobial resistance. Infect Dis 1997;4:
9. Murray BE, Alvarado T, Kim KH, Varachit M, Jayanetra P, Levine MM et al. Increasing resistance to trimethoprim-sulfamethoxazole among isolates of *Escherichia coli* in developing countries. J Infect Dis 1985;147:724-8.
10. Guyon A, Barman A, Ahmed JU, Ahmed AU, Alam M. A baseline survey on use of drugs at primary health care level in Bangladesh. Bull World Health Organ 1994;72:265-71.
11. Rosmans C, Islam T, Bennish ML. Medical practitioners' knowledge of dysentery treatment in Bangladesh. BMJ 1996;313:205-6.
12. Thamlikitkul V. Antibiotic dispending by drug store personnel in Bangkok, Thailand. J Antimicrob Agent Chemother 1988;21:125-31.

13. Hossain MM, Glass RI, Khan MR. Antibiotic use in a rural community in Bangladesh. Int J Epidemol 1982;11:402-405.

14. Goel P, Ross-Degnan D, Berman P, Soumerai S. Retail pharmacies in developing countries: a behavior and intervention framework. Soc Sci Med 1996;42:1155-61.

15. Shahid NS, Rahaman MM, Hairder K, Banu H, Rahman N. Changing pattern of resistant *Shiga bacillus* (*Shigella dysenteriea* type 1) and *Shigella flexnerii* in Bangladesh. J Infect Dis 1985;152:1114-9.

16. Hakk H, Hardon AP. Indigenised pharmaceutics in developing countries: widely used, widely neglected. The Lancet 1988;2:620-1.

17. Reyes H, Guiscafre H, Munoz O, Perez-Cuevas R, Martinez H, Gutierrez G. Antibiotic non-compliance and waste in upper respiratory infections and acute diarrhea. J Clin Epidemol 1997;50:1297-304.

18. Lansang MA, Lucas-aquino R, Tupasi T, Mina VS, Salaza, LS, Joban N et al. Purchase of antibiotics without prescription in Manila, the Philippines. Inappropriate choice and doses. J Clin Epidmiol 1990;43:61-7.

19. Esezobo E, Offiong E. In vitro studies on some brands of oxytetracycline capsules available in Nigeria. Nigerian J Pharmacol 1986;17:24-8.

20. Taylor RB, Shakoo, O, Behrens RH. Drug quality, a contributor to drug resistance? The Lancet 1995;346:122.

21. Calva JJ, Sifuentes-Osornio J, Ceron C. Antimirobial resistance in fecal flora: longitudinal community-based surveilence of children from urban Mexico. Antimicrob Agents Chemother 1996;40:1699-702.

22. Lamikanra A, Fayinka ST, Olusanya OO. Transfer of low-level trimethoprim resistance in faecal isolates obtained from apparently healthly. Nigerian students FEMS Microbial Lett 1989;5:275-8.

23. Lamikanra A, Okeke IN. A Study of the effect of the urban / rural divide on the incidence of antibiotic resistance in *E.coli*. Biomedical Letters 1997;55:91-7.

24. Korte R, Rehle T, Merkle A. Strategies to maintain health in the third world. Trop Med Parasitol; 42:428-32.

25. Okello D, Konde J, Lubanga R, Arube-Wani J. Waste disposal in private medical clinics in Kampala, Uganda. J Clin Epidemiol 1997; 50: Suppl. A1:45s.

26. Brown RC. Antibiotic sensitivity testing for infections in developing countries: lacking the basis [letter]. JAMA1996;276:952-3.

27. Ringertz S, Muhe L, Karantz I, Hathaway A, Shamebo D, Oferij L et al. Prevalence of potential respiratory disease bacteria in children in Ethiopia. Antimicrobial susceptibility of pathogens and use of antibiotics among children. Acta Paediatr 1993;82:843-8.

28. Rahal K, Wang F, Schindler J, Rowe B, Cookson B, Huovinen P et al. Report on surveillance of antimirobial resistance in individual countries. Clin Infect Dis 1997;24:Suppl.S169-75.

29. Mutada LN, Omari AM, Wamola IA. Adaptation of a method of measuring zone diameters of bacterial growth inhibition by suit developing countries. East Afr Med J 1998;66:441-7.

30. Van den Bogaard AE. Antimicrobial resistance - relation to human and animal exposure to antibiotics. J Antimirob Chemother 1997;40:453-4.

31. Union of Concerned Scientists. Food and environment, programme overview focus on antibiotic resistance. Available from:
URL: http://www.ucsusa.org.

32. Stefan S, Noble WC. Aspects of bacterial resistance to antimicrobials used in veterinary dermatological practice. J Vet Dermatol 1999;10:63-76.

33. Wise R, Hart T, Cars O, Streulens M, Helmuth R, Huovinen P et al. Antimicrobial resistance is a major threat to public health. BMJ 1998;317:609-610.

34. Cook RR. Atimirobial resistance - use in veterinary and human medicine. J Antimicrob Chemother 1997;39:435.

35. Piddock LJV. Does the use of antimicrobial agents in veterinary medicine and animal husbandry select antibiotic resistant bacteria that infect man and compromise antimicrobial chemotherapy? J Antimicrob Chemother 1996;38:1-3.

36. CDC, National Antibiotic Resistance Monitoring System Frequently asked question about: antibiotic resistance and foodborne illness. Available from: URL: http://www.cdc.gov/narms/faq.htm.

37. Barza M. Potential mechanisms of increased disease in humans from antimicrobial resistance in food animals. Clin Infect Dis 2002; 34:Suppl.3:S123-5.

38. Moreno MA, Dominguez L, Teshager T, Herrero IA, Porrero MC. Antibiotic resistance monitoring: the Spanish programme. Int J Antimicrob Agents 2000;14:285-9.

39. World Bank Group: Data and statistics. Available from:
URL: http://www.worldbank.gov/data/

40. Cornwall J. *Tuberculosis*: a clinical problem of international importance. The Lancet 1997;350:660-1.

41. CIA. The World Fact Book. Available from:
URL: http://www.cia.gov/cia/publications/factbook/geos/vm.html.

42. UNFPA. Viet Nam background information. Available from:
URL: http://www.unfpa.org/regions/apd/countries/Viet Nam.htm.

43. Sophie. "Doi Moi and health: The effect of economic reforms on the health system in Viet Nam". Internat J Health Planning Manag 1996;11:159-72.

44. Amsterdam: Royal tropical Institute. KIT and Hanoi School of Public Health: a study on motivation of health staff in northern Viet Nam. 2001.

45. The Health Statistics Division of the Planning Department of the Ministry of Health, Ha Noi, Viet Nam. Health Statistics Yearbook 2001

46. Falkenberg T, Nguyen TB, Larsson M, Nguyen TD, Tomson G. Pharmaceutical sector in transition: a cross sectional study in Vietmam, Southeast Asia. J Trop Med Public Health 2000;31:590-7.

47. Okumura J, Wakai S, Umenai T. Drug utilisation and self-medication in rural communities in Viet Nam. Soc Sci Med 2002;54:1875-86.

48. Toan NV, Trong LN, Hojer B, Persson LA. Public health services use in a mountainous area, Viet Nam: implications for health for policy. Scan J Public Health 2002;30:86-93.

49. Unpublished information of enteric infectious diseases from National Institute of Hygiene and Epidemiology Hanoi, Viet Nam.

50. Wain J, Hien TT, Connerton P, Ali T, Parry CM, Chinh NT et al. Molecular typing of multiple-antibiotic-resistant *salmonella enterica* serovar typhi from Viet Nam: application to acute and relapse cases of typhoid fever. J Clin Microbiol 1999;37:2466-72.

51. Connerton P, Wain J, Hien TT, Ali T, Parry C, Chinh NT et al. Epidemic typhoid in Viet Nam: molecular typing of multiple-antibiotic-resistant *salmonella enterica* serotype typhi from four outbreaks. J Clin Microbiol 2000;38:895-7.

52. Tran HH, Gunnar B, Nguyen BM, Guerin P. The risk factors associated with typhoid fever in Sonla province northern Viet Nam. The thesis of Master of Philosophy in International Community Health 2003.

53. Hoa NT, Diep TS, Wain J, Parry CM, Hien TT, Smith MD et al. Community acquired septicaemia in southern Viet Nam: the importance of multidrug-resistant Salmonella Typhi. Trans R Soc Trop Med Hyg 1998;92:503-8.

54. Edwards PR, and Ewing WH. Identification of Enterobacteriaceae. 3rd ed. Minneapolis: Burgress Publishing Co. 1972.

55. Levine MM. Escherichia coli that cause diarrhea: enterotoxogenic, enteropathogenic, enteroinvasive, enterohemorrhagic and enteroadherent. J Infect Dis 1987;155:377-89.

56. Maps online resource. Bac Ninh Map. Available from:
URL: http://basao.com.vn/map/vn_dolmap/

57. Bac Ninh portal. Available from: URL: http://www.Bac Ninh.gov.vn/

58. Smith PG, Morrow RH. Method for field trial of intervention against tropical disease: a “toolbox”. Oxford, New York, Tokyo: Oxford University press,1998.

59. Altman DG. Comparing group - continuous data. In: Practical statistics for medical research. London, Glasgow, Weinheim, New York, Tokyo, Melbourne, Madras: Chapman & Hall, 1991. p. 179-223

60. Swinscow TDV. revised by Cambell MJ. Statistic at square one. Medical statistics made as simple as possible. London: BMJ publishing group, 1996.

61. Armitage P, Berry G. Statistical method in medical research. A full and explicit reference work on statistics. Oxford: Blackwell, 1994.

62. Charles HH Julie EB. Epidemiology in medicine. Wolters Kluwer Company, USA, 1987

63. Altman DG. Practical statistics for medical research. First ed. New York: Chapman and Hall, 1999.

64. Thomas CT. An introduction to epidemiology. Third ed. Jones and Bartlett publishers; 2002.

65. Marshall C, Rossman BG. Designing qualitative research. Second ed. SAGE Publication: 1999.

66. World Health Organisation. A manual for the treatment of diarrhea. World Health Organisation: Geneva, Switzerland, 1990. p. 6-7.

67. Edwards PR, Ewing W H. Identification of *Enterobacteriaceae*. 3rd ed. Minneapolis: Burgress Publishing Co., 1972.

68. Levine MM. *Escherichia coli* that cause diarrhea: enterotoxogenic, enteropathogenic, enteroinvasive, enterohemorrhagic and enteroadherent. J Infect Dis 1987;155:377-89.

69. Okumura J, Wakai S, Umenai T. Drug utilisation and self-medication in rural communities in Viet Nam. Soc Sci Med 2002;54:1875-86.

70. The Swedish reference group for antibiotic. A revised system for antibiotic sensitivity testing. Scan J Infect Dis 1981;13:148-52.

71. National Committee for Clinical Laboratory Standard. Zone diameter interpretative standards and equivalent minimum inhibitory concentration according to standard M100-S5 table M2-A5. National committee for Clinical Laboratory Standard. Villanova. Pa 1994.

72. Wain J, Hien TT, Connerton P, Ali T, Parry CM, Chinh NT et al. Molecular typing of multiple-antibiotic-resistant *Salmonella Enterica* serovar typhi from Viet Nam: application to acute and relapse cases of typhoid fever. J Clin Microbiol 1999;37:2466-72.

73. Nataro JP, Kaper JB. Diarrheagenic Escherichia coli. Clin Microbiol Rev 1998;11:142-201

74. Muruleedhara B, Melanie F, Dawn S, Richard W. Ubiquity and persistence of Escherichia coli in a Midwestern coastal stream. Appl Envir Microbiol 2003;69: 4549-55

75. Timothy RD, Helena MG, Carol JP. Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. Appl Envir Microbiol 2002;68: 1165-1172

76. Helena MG, Melinda AW, Timothy R D, Carol JP. Sources of Escherichia coli in a coastal subtropical environment. Appl Envir Microbiol 2000;66: 230-7.

77. Richard LW, Meredith BN. Foreshore sand as a source of escherichia coli in Nearshore water of a Lake Michigan beach. Appl Envir Microbiol 2003;69: 5555-62

78. Occupational and environmental health. Available from URL: http://www.link.med.ed.ac.uk/hew/info

79. Vella V. Epidemiological estimates of infectious diseases in Kwazulu-Natal. Kwazulu-Natal. Epidemiology Bulletin. March 2003.

80. Gross RJ, Rowe B. Escherichia coli diarrhoea. J Hyg, 1985;95:531-50.

81. Hoge CW, Gambel JM, Srijan A, Pitarangsi C, Echeverria P. Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. Clin Infect Dis 1998;26:341-5.

82. Hart CA, Kariuki S. Antimicrobial resistance in developing countries. Br Med J 1998;317:647-50.

83. Levy S, Marshall B, Schleuderberg S, Rowe B, Davis J. High frequency of antibiotic resistance in human fecal flora. Antimicrob Agents Chemother 1988;32:1801-6.

84. Levin B, Lipsitch M, Perrot V, Schrag S, Antia R, Simonsen L, et al. The population genetics of antibiotic resistance. Clin Infect Dis 1997;24:S9-16.

85. Levy SB. Antibiotic resistance: an ecological imbalance. Ciba Found Symp 1997;207:1-9; discussion 9-14.

86. Jordi V, Martha V, Climent C, Honorato U, Hassan M, David S, et al. Antimicrobial resistance of diarrheagenic Escherichia coli isolated from children under the age of 5 years from Ifakara, Tanzania. Antimicrob Agents Chemother 1999;43:3022-4.

87. David GW, Patricia AB. Expanded-spectrum cephalosporin resistance in E.coli isolates associated with bovine calf diarrheal disease. J Antimicrob Chemother 1999;44:607-10.

88. Okeke IN, Fayinka ST, Lamikanra A. Antibiotic resistance trends in Escherichia coli from apparently healthy Nigerian students (1986-1998). Emerg Infect Dis 2000;6:393-6.

89. Subhra C, Deokule JS, Pallavi G, Bhattacharya SK, Nandy RK, Nair GB, et al. Concomitant infection of enterotoxigenic Escherichia coli in an outbreak of cholera caused by Vibrio cholerae O1 and O139 in Ahmedabad, India. J Clin Microbio 2001;39:3241-6.

90. Nys S, Okeke IN, Kariuki S, Dinant GJ, Driessen C, Stobbringh EE. Antibiotic resistance of faecal *Escherichia coli* from healthy volunteers from eight developing countries. J Antimicrob Chemother. 2004;

91. Chuc NT, Tomson G. “Doi moi” and private pharmacies: a case study on dispensing and financial issues in Hanoi, Vietnam. Eur J Clin Pharmacol. 1999;55:325-32.

APPENDICES

Appendix 1: Ethical clearance

UNIVERSITETET I BERGEN
Det medisinke fakultet
Harald Hårfagregst. 1,
Postboks 7800, 5020 BERGEN
Tlf: 55 58 20 84/86
Fax: 55 88 96 82
E-post: Rek-3@uib.no

UINVERSITY OF BERGEN
Faculty of Medicine
Harald Hårfagregst. 1,
PO box 7800, N-5020 BERGEN
Ph: +47 55 58 20 84/86
Fax: +47 55 88 96 82
E-mail: Rek-3@uib.no

http://www.etikkom.no/REK/

Regional komité for
medisinsk forskningsetikk
Vest-Norge (REK Vest)

Bergen, 28.06.04
Sak nr.04/

To whom it may concern

Confirmation (REK Vest no. 118.04)

We hereby confirm that the research protocol *The Leading Risk Factors in Acquired Bacterial Resistance to Antibiotics in the Rural Areas of Bac Ninh Province, Northern VIET NAM,* by *Ngo Tuan Cuong* (student), *Gunnar Bjune* (supervisor), *Preben Aavistland* (supervisor), and *Nguyen Binh Minh* (supervisor),has been evaluated by The Regional Committee for Medical Research Ethics in Western Norway (REK Vest).

The protocol is cleared.

Sincerely,

Arne Salbu
Secretary

(Signed)

Appendix 2: **QUESTIONNAIRES**

Code: ☐☐☐

Please, fill in the appropriate square box

I. PERSONAL DEMOGRAPHIC INFORMATION

1. Name: . . . . . .
2. Sex: Male ☐ 1 Female ☐ 2
3. Age: . . . . . Date of birth: . . . . . / . . . . . / . . . . . .

   Name of father/mother (for children under 5) : . . . . . .
4. Address: . . . . . .
5. Marital status:

   Single: ☐ 1

   Married: ☐ 2

   Divorced: ☐ 3

   Widowed: ☐ 4

   Separated: ☐ 5
6. Ethnic group:

   Kinh: ☐ 1

   Tay: ☐ 2

   Nung: ☐ 2

   Other: ☐ 2
7. Religion:

   Buddhism: ☐ 1

   Christian: ☐ 2

   Other (religious community) ☐ 3

   None: ☐ 4
8. Level of education:

   Illiterate: ☐ 1

   Primary school: ☐ 2

Secondary school: ☐ 3

Comprehensive school: ☐ 4

University: ☐ 5

9. Occupations

Civil servant, teacher, officer: ☐ 1

Industry worker: ☐ 2

Farmer, fisherman: ☐ 3

Health worker: ☐ 4

Housewife: ☐ 5

Student: ☐ 6

Other: ☐ 7

II. CLINICAL DATA

10. Weight: . . . . .
11. Height: . . . . .
12. Hospitalised on: . . . . . / . . . . . / . . . . . .
13. Clinical signs

Fever (higher than 38^{O}C): Yes ☐ 1 No ☐ 2

If yes: Temperature: . . . . . ^{O}C

Number of days with fever: . . . . . .

Headache Yes ☐ 1 No ☐ 2

Vomit Yes ☐ 1 No ☐ 2

Diarrhoea Yes ☐ 1 No ☐ 2

If yes: Number of diarrhoeal movements per day: . . . . .

Number of days with diarrhoea: . . . . . .

Treatment before hospitalisation: . . . . . .

. . . . . .

Treatment during hospitalisation: . . . . . .

. . . . . .

14. Date of discharge: . . . . . / . . . . . / . . . . . .

15. Is there any complication? Yes ☐ 1 No ☐ 2

If yes, describe: . . . . . .

. . . . . .

. . . . . .

Laboratory information.

Confirmed Yes ☐ 1 No ☐ 2

Positive with EPEC Yes ☐ 1 No ☐ 2

Isolated cultured: . . . . . .

Lab: . . . . . .

Specimens date: . . . . . / . . . . . / . . . . . .

Serotype: . . . . . .

III. RISK FACTOR ASSOCIATED WITH THE MEDICAL SITUATION

16. Chronic illness

Yes ☐ 1 No ☐ 2

If yes, list: 1 - . . . . . .

2- . . . . . .

3 - . . . . . .

17. Recent hospitalisation

Yes ☐ 1 No ☐ 2

18. Recent out patient clinic visit

Yes ☐ 1 No ☐ 2

19. History of diarrhoea in last 90 days

Yes ☐ 1 No ☐ 2

20. Personal history to antibiotic treatment in last 90 days

Yes ☐ 1 No ☐ 2

21. Recent history to visit another patient in hospital or clinic

Yes ☐ 1 No ☐ 2

22. Recent contact with diarrhoeal patient

Yes ☐ 1 No ☐ 2

IV. ANTIBIOTIC CONSUMPTION

23. Where do you go when you are getting sick, especially when have diarrhoea?

Go to hospital	☐ 1
Go to private clinic	☐ 2
Go to drugstore	☐ 3
Self-treatment	☐ 4
Go to traditional healer	☐ 5

24. According to you, what are the sign of diarrhoeal diseases caused by infectious?

Fever	☐	Vomit	☐
Headache	☐	Nose-run	☐
Diarrhoea	☐	Swell and hurt	☐
Tired	☐	Loss weighs	☐

25. What type of drugs does you often used when you are getting sick?

Antibiotic	☐ 1
Tonics	☐ 2
Flu table	☐ 3
Traditional medicine	☐ 4
Other	☐ 5

26. Do you often use antibiotic?

Yes ☐ 1 No ☐ 2

27. If antibiotic, where did/do you buy it?

In the private pharmacy	☐ 1
In the street vendor	☐ 2
In the hospital pharmacy	☐ 3

28. Do you often buy antibiotic with or without prescription of medical doctor?

Yes ☐ 1 No ☐ 2 (if yes, move to 30th)

29. Who did/do you consult to buy and use them?

Advice from drug seller ☐ 1

Decided yourself ☐ 2

Advice from neighbourhood ☐ 3

30. Do you think that it is easy to buy antibiotics in your community?

Yes ☐ 1 No ☐ 2

31. When did/do you often use an antibiotic?

Infection ☐ 1

Flu ☐ 2

Headache ☐ 3

Diarrhoea ☐ 4

Pain ☐ 5

Other ☐ 6

32. How do you use antibiotics?

One kind of antibiotic ☐ 1

Combination ☐ 2

33. If it is combination, how many kinds of antibiotic do you often use?

2 kinds of antibiotics ☐ 1

3 kinds of antibiotics ☐ 2

More ☐ 3

34. Which way do you like to use antibiotic?

Injection ☐ 1

Oral ☐ 2

Other ☐ 3

35. What types of antibiotic do you often use when get diarrhoea (please specify)?

. . . . . .

. . . . . .

36. If you use antibiotic, for how long?

Stop after reduced symptoms	☐ 1
Two days	☐ 2
Four days	☐ 3
One week	☐ 4
Two weeks	☐ 5

37. What do you think about the effect of antibiotics in treatment?

Infections diseases	☐ 1
All types of disease	☐ 2

38. Do you often keep some antibiotics in your family?

Yes ☐ 1 No ☐ 2

39. What do you think about the price of antibiotics?

Expensive	☐ 1
Medium	☐ 2
Acceptable	☐ 3

40. If you do not have enough money to buy the drug following the doctor's prescription, what would you do?

Reduce dose	☐ 1
Choose another one that is cheaper	☐ 2
Borrow more money	☐ 3

V. RISK FACTOR ASSOCIATED WITH THE LIVING AREA

41. Type of water supply in the family

Municipal water supply	☐ 1
Wells	☐ 2
Pond	☐ 3
Rainfall water	☐ 4

42. How did you drink water?

Drinking boiled water ☐ 1

Drinking un-boiled water ☐ 2

43. How did you often eating outside?

Yes ☐ 1 No ☐ 2

44. Type of toilet in your family

Model toilet ☐ 1

Hygienic latrine ☐ 2

Unhygienic latrine ☐ 3

Excrete direct in to fishpond or environmental ☐ 4

45. Did you often use a public sewage system in your living area?

Yes ☐ 1 No ☐ 2

VI. FAMILY ECONOMIC LEVEL

46. How many people in your family: . . . . .

47. What kind of items do you have in you family

Motorcycle ☐ Radio ☐

Bicycle ☐ Camera ☐

Television ☐ Other: . . . . . .

48. How many of each of the items on the list here does your family have?

Cow: . . . . . Pig: . . . . . .

Buffalo: . . . . . Chicken: . . . . . .

49. How much money does your family earn every month: . . . . . .

50. Do you have electricity at home

Yes ☐ 1 No ☐ 2

Thank you very much for your collaboration.

DATE: . . . . ./. . . . ./ 2004

Interviewer's name: . . . . . .

Appendix 3: Consent form

CONSENT FORM

My name is . . . . . and I am a student at the University of Oslo, Norway carrying out a research project to find the leading risk factors which associate to antibiotic resistance in rural areas of North Viet Nam.

I am inviting all the diarrhoeal patients admitted to the Bac Ninh hospital (from August 2004 to December 2004) to join a study and answer some questions (according to the questionnaires). All your answerer will help me to find out the risk factors which influence to the antibiotic resistance, a big problem in our country.

It is very important that you understand that everything you say will be completely confidential, and your name will not appear on any publicly seen document. Also, you may stop the interview at any time if you do not wish to continue.

If you agree to participate you must give me your name and address. I am the only person who will ever see this list.

One time again, your participation in this study will help policy maker to know about the reasons of antibiotic resistant situation in this areas and they can have the suitable methods to reduce this situation. It will make the treatment for the patient more effect in the future.

Do you have any questions?

Will you participate in this study? ? (Yes)

? (No)

Signature

Appendix 4: Gantt chart